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ELECTRONIC TECHNIQUES IN MEDICINE

THE EIGHTH EDSEL B. FORD LECTURE, 1959

VLADIMIR K. ZWORYKIN, PhD.*

Less than four months ago some 500 delegates from 15 countries gathered in Paris for the Second International Conference on Medical Electronics and founded the International Federation of Medical Electronics. Some 130 technical papers were presented at the Conference which, for a period of four days, held its meetings at the UNESCO headquarters. Plans were laid for a similar meeting in 1960 in London, with the prospect of a further meeting in 1961 in Moscow.

These are hopeful signs that medical electronics is at last achieving the wide recognition which this powerful tool for the advancement of medical knowledge and the conquest of disease merits. And yet, it is as true as ever that medical electronics lags far behind its potentialities.

The reasons for this are more readily recognized than overcome. They are partly a matter of communication, partly one of economics. Thus, success in medical electronics depends on the close cooperation of electronic engineers and medical men. If the engineer is to contribute his share to the common effort he must not only be proficient in his own field, but must also know enough of medicine to be able to communicate with medical men and life scientists so as to learn of their problems and difficulties. Conversely, the medical man or biological investigator must learn enough electronics to collaborate effectively with electronic engineers and instrument designers. Specialized institutes and medical engineering curricula have been established in recent years to deal with this problem. These represent a valuable contribution. However, their scope must be greatly extended to meet the existing need.

The economic difficulty proceeds from the entirely proper conservatism of the medical profession in its approach to new techniques. New types of medical equipment find general acceptance only after exhaustive clinical tests. Consequently, there is a long delay between the original engineering investment and an adequate financial return from the sale of the finished article. The result is a natural reluctance on the part of instrument manufacturers to undertake the development of new types of medical apparatus. This obstacle to progress in medical electronics might be overcome if government or philanthropic agencies could be persuaded to absorb development and testing costs.

The above difficulties are very real. However, the challenge of adapting electronic techniques to the needs of medicine in the interest of the prevention or relief of human suffering has been such that considerable headway has been made in the face of them. Today, I want to describe briefly some of these techniques and point out likely avenues of further advance.

The fact that every vital process is accompanied by an electrical change constitutes a direct invitation for the development of refined electrical measuring techniques as aids in physiological research and diagnosis. Such techniques have been carried farthest for the study of the activities of the heart and the brain. Indeed, Einthoven's

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string galvanometer was developed expressly for the measurement of heart-muscle potentials or electro-cardiography long before the advent of electronics. It has generally been replaced by instruments with electronic amplification which serve, at present, for the diagnosis of heart conditions as well as for the localization of brain tumors and the indication of epilepsy.

The record of the conventional electrocardiograph or electroencephalograph consists of a series of curve tracings representing the time variation of the potential difference between fixed points on the body surface. Its interpretation demands familiarity with the conventional placement of the electrodes and the corresponding sequence of the tracings. A much more direct visualization of electrical processes on the body surface, elicited by activity at the heart or brain, is permitted by so-called area displays. Different types of area displays have been developed by Stanford Goldman[1] and his associates in the Massachusetts Institute of Technology, by Harold Shipton[2] at the Burden Institute in England, by J. C. Lilly[3] at the National Institutes of Health, and by Y. N. Ananiev[4] in Russia. In Lilly's apparatus (Fig. 1) a rectangular array of electrodes attached to the exposed cortex of a cat is connected with a similar

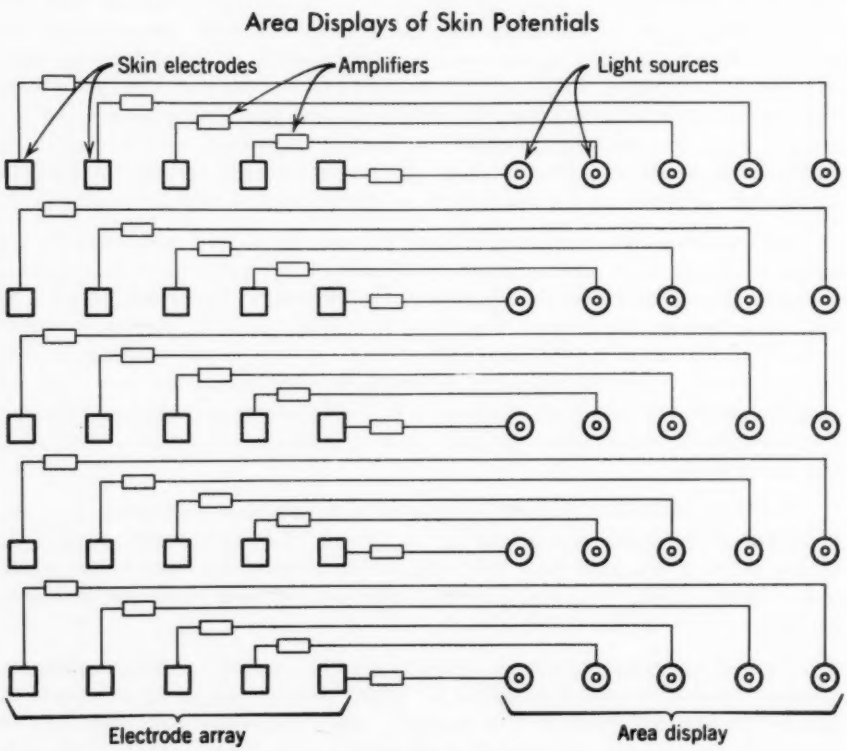


Figure 1
Area Display of Skin Potentials.

array of indicator tubes, whose brightness is modulated by the potential of the corresponding electrode. The indicator panel is photographed by a high-speed motion picture camera and the film reprojected at a lower speed. With this apparatus it is found that various stimuli produce characteristic sweeps of potential across the panel. While limited time and space resolution, improved to some extent in later instruments, called for further apparatus development, the technique constitutes an effective tool for the study of the nervous system and the localization of defects in the brain.

Electronic techniques are not limited to a purely passive role in the investigation of the nervous system. As Wilder Penfield[5] of the Montreal Neurological Institute has shown, the application of appropriate stimuli to different parts of the cortex can cause recollection of remote memories. This represents a first step in an experimental approach to the problem of memory storage. Other electrical techniques for the active control of the nervous system have been proposed for non-convulsive electroshock therapy for mental disorders and for sleep therapy. Both of these techniques are assumed to function by blocking normal cortical conduction. Further investigation may be required to establish definitely whether the Russian "Electrosonne" apparatus, developed for producing sleep in patients suffering form insomnia, owes its effectiveness to direct physiological action or rather to suggestion.

A much more radical intervention in the nervous system is realized in brain surgery, which also can be greatly aided by electronics. It has been found that, in certain disease conditions, relief can be obtained by the destruction of specific neural tissues. These may lie deep within the brain, at or beyond the limits of conventional surgery. In such cases, focused beams of ultrasound have been found helpful in destroying tissue at the beam focus without injury to the intervening layers. In the equipment developed by W. J. Fry of the University of Illinois, four 1-mc sound beams are focused by polystyrene lenses at a carefully predetermined common point within the patient's skull, which is perforated to provide entrance ports for the beams.[6] (Fig. 2). This technique has been employed successfully in the treatment of Parkinson's disease.

In the case of heart disease, too, the role of electronics is not limited to diagnosis. A well-established technique to overcome heart standstill, encountered from time to time in the course of surgical operations, is to apply externally pacemaker electrical pulses with the normal heart rhythm. If the heart condition which leads to standstill is momentary, this technique is entirely satisfactory, since normal heart action will be resumed after a very brief application of the pulses. On the other hand, if the heart condition is chronic it ceases to be practical since the pacemaker pulses result in undesirable stimulation of other muscle tissue. Furthermore, the direct insertion in the heart muscle of the stimulating electrodes attached to an external pulse generator proves unsatisfactory, since the penetration of the electrodes through the skin creates a source of irritation and infection. This difficulty was finally removed by replacing the direct coupling of the electrodes to the pulse generator by inductive coupling and surgically inserting the plastic-coated secondary of the output transformer of the pulse generator under the skin of the patient. Fig. 3 shows the insert developed by Dr. Alexander Mauro of the Rockefeller Institute. In due time, the application of

transistor techniques and compact batteries should lead to portability of the entire pulse generating system, so that the patient may move about freely.

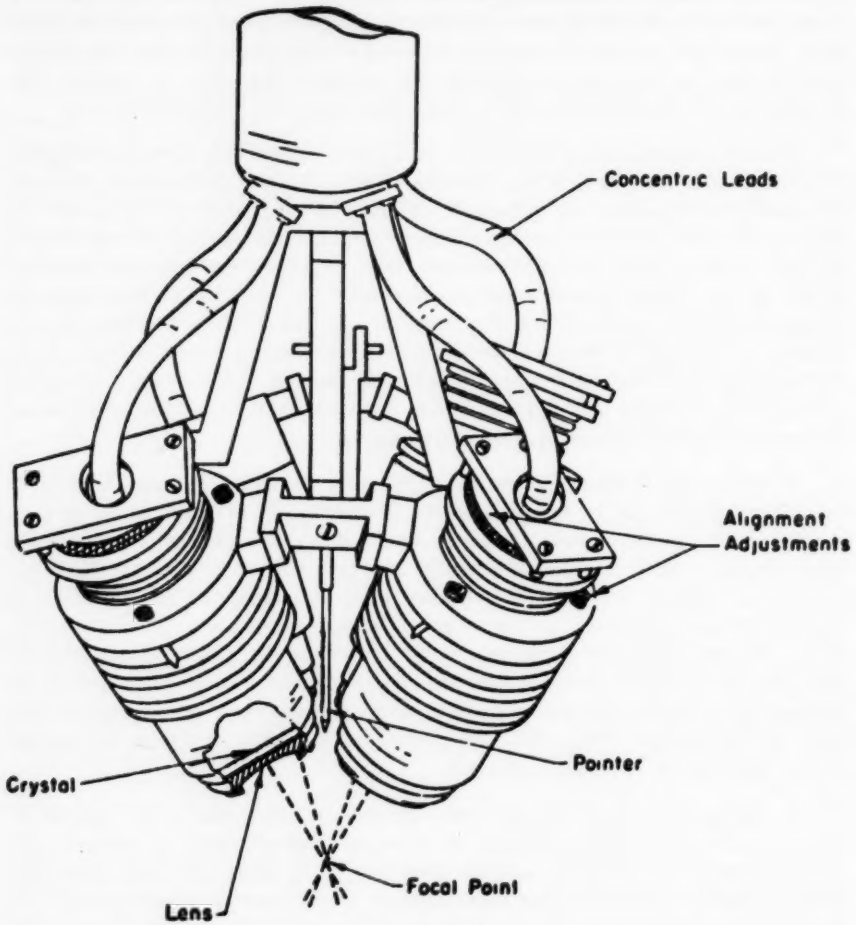


Figure 2
Ultrasonic Focusing Irradiator after W. J. Fry (W. Welkowitz, reference 6).

New approaches for the study of the heart and brain have been supplemented by new techniques for the investigation of the gastro-intestinal tract. The new tool provided by electronics for this purpose is the endoradiosonde or "radio pill" which has been developed in various forms by the Medical Electronics Center of the Rockefeller Institute in cooperation with Dr. J. T. Farrar[7] of the Veterans Administration Hospital in New York and RCA's Industrial Electronic Products Division; by Stuart Mackay and Bertil Jacobson[8] in Sweden; by M. v. Ardenne[9]; and H. B. Srung in Dresden; and by H. G. Noeller in Heidelberg. Our pill (Fig. 4) consists

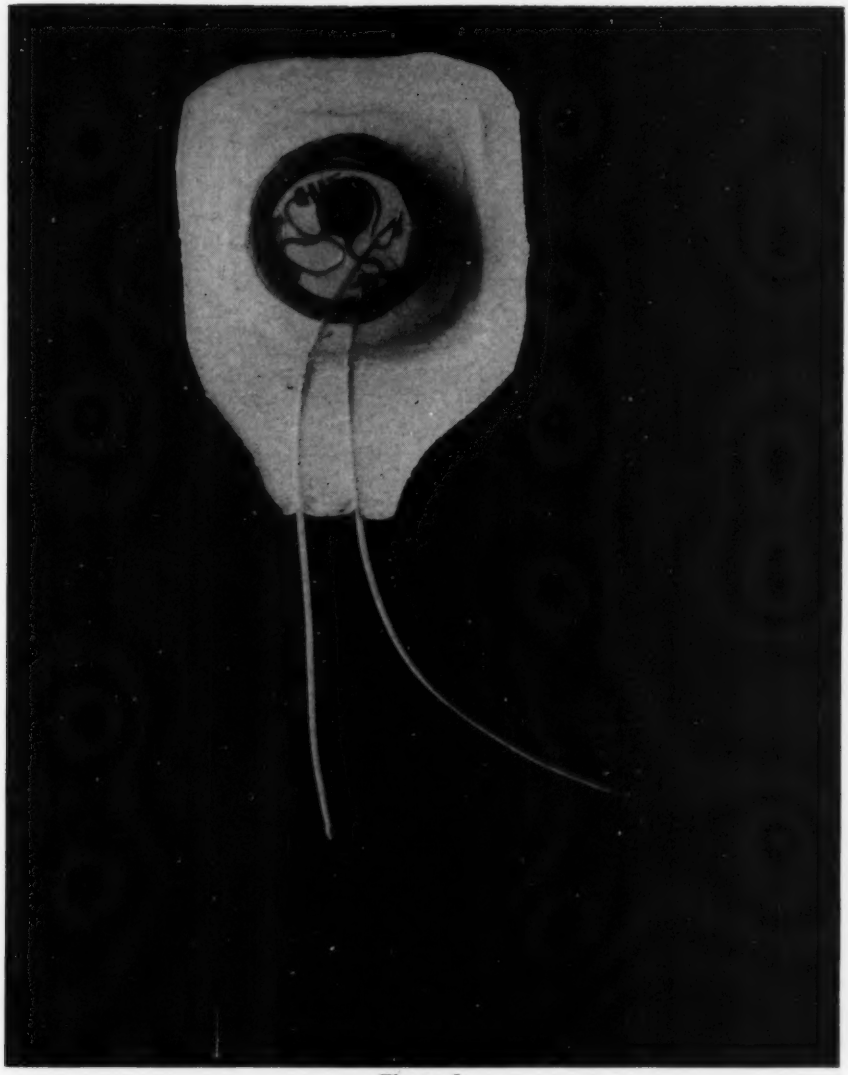


Figure 3

Heart Muscle Stimulator. The part shown is inserted surgically and coupled electromagnetically to the external power source. (Courtesy of Dr. Alexander Mauro).

essentially of a minute transistor oscillator whose frequency is modulated by small pressure changes and whose leakage field is detected by a frequency-modulation detector at a distance of the order of a foot away (Fig. 5). The pressure-sensitive element is a flexible diaphragm supporting the armature of the circuit inductance.

All these components are quite conventional. The special problem to be solved

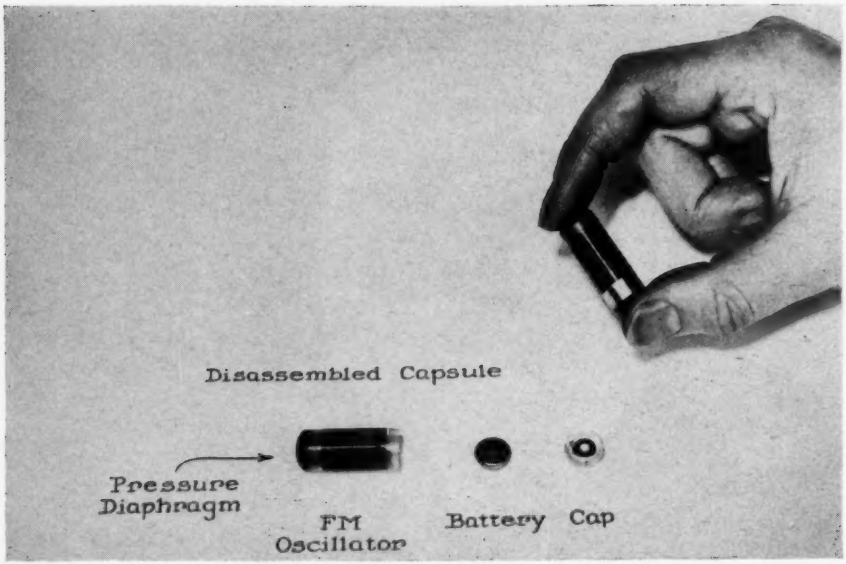


Figure 4
The Radio Pill.

before the radio pill could become a practical tool for the study of the gastro-intestinal tract was to encapsulate the oscillator in a passive envelope small enough to be swallowed without discomfort. This envelope had to include a power source sufficient to maintain oscillations for the time of passage through the tract — from one to several days. The actual pill is about 1-inch in length and 0.4 inch in diameter. The firm plastic envelope is perforated and covered by a membrane at one side to communicate pressure variations to the interior. Power is provided by a 1.2 volt rechargeable cadmium battery. The pressure variations are registered by a recording galvanometer attached to the frequency modulation detector. Studies on 28 patients have shown that the radio pill does not affect digestive processes and is not a source of discomfort. It can thus be employed for patients too ill to permit the use of more conventional techniques for the study of portions of the tract, such as gastroscopy.

We now turn away from techniques concerned with diagnosis and treatment of a particular part of the human anatomy to medical tools of a more general nature. One of the most powerful of these is the use of x-rays for diagnosis. While the use of x-rays for this purpose has no rival in many applications, we have become increasingly aware of the damage which may result from excessive x-ray exposure. This danger exists particularly in direct fluoroscopic examinations since, in conventional fluoroscopy, the light emitted by the x-ray screen is so inefficiently utilized by the observer's eye that the discrimination of sufficient detail may demand exposure to levels of radiation a thousand times more intensive than those required for x-ray

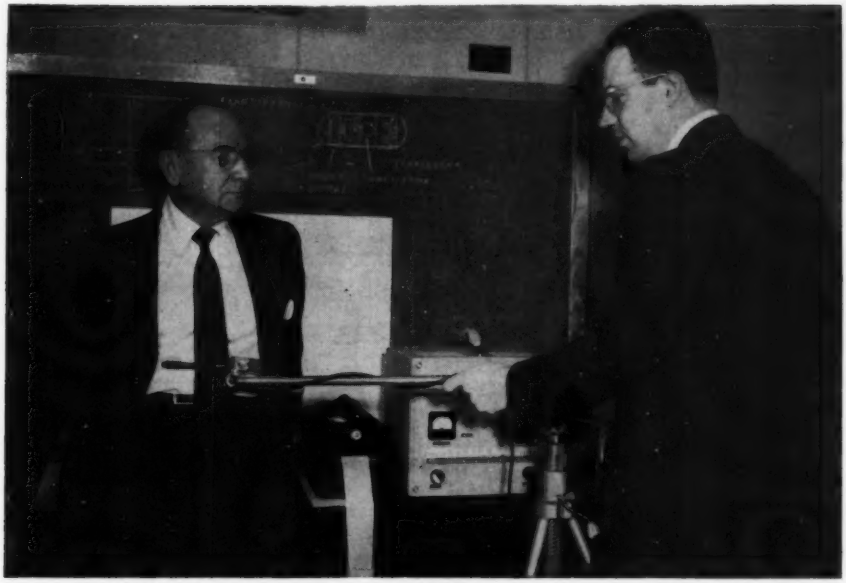


Figure 5
Signal Pickup with the Radio Pill.

photography. Fortunately, electronic amplification can raise the brightness level of the observed image by a comparable factor and thus reduce the necessary x-ray exposure of the patient to relatively safe levels.

Present methods of electronic image amplification are largely borrowed from television practice. In fact, one of the most flexible techniques employs simply a very-large-aperture lens to image the conventional fluoroscopic screen on the photocathode of a high-sensitivity image orthicon camera.[10] (Fig. 6.) The image is viewed on the screen of a television monitor at a light level which is essentially independent of the screen brightness. In another system, the x-rays impinge on a fluorescent screen in intimate contact with the photocathode of an image tube and the photoelectrons emitted by the cathode are accelerated and focused on a small fluorescent screen. The small screen is then observed directly with a magnifier, or indirectly with a compact television chain.[11] (Fig. 7.) The acceleration of the electrons and the size reduction of the image combine to yield an increase in brightness of the viewing screen over the x-ray screen approaching a thousand.

Still another system, which is perhaps the most interesting, but least developed of the three, employs a photoconductive layer in series with an eletroluminescent film.[12]. (Fig. 8.) Alternating voltages are applied across a sandwich of the two films, and the x-ray pattern is projected on the photoconductor. Light is emitted by those portions of the electroluminescent layer which are backed by photoconductor subjected to x-ray radiation; the x-rays increase the conductivity of the photoconductor, effectively short-

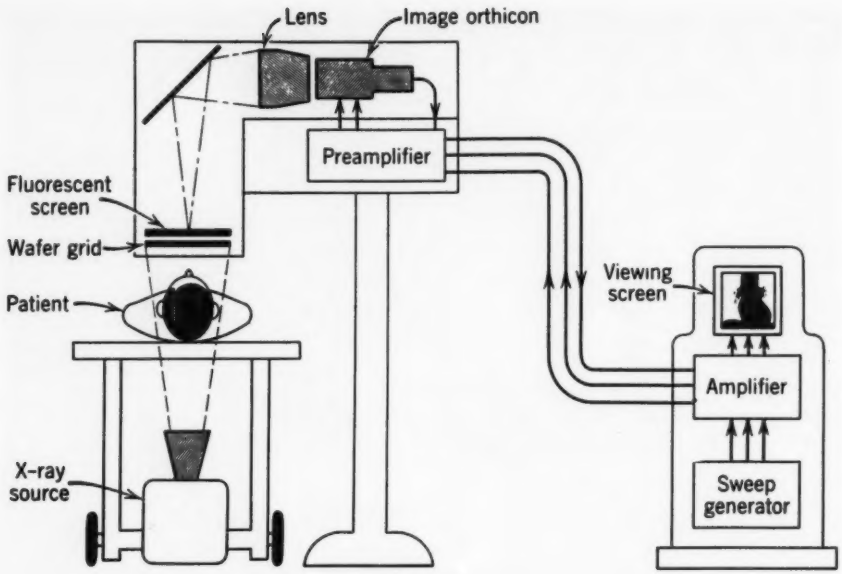


Figure 6
Television System for Observing X-Ray Screen (Morgan, reference 10).

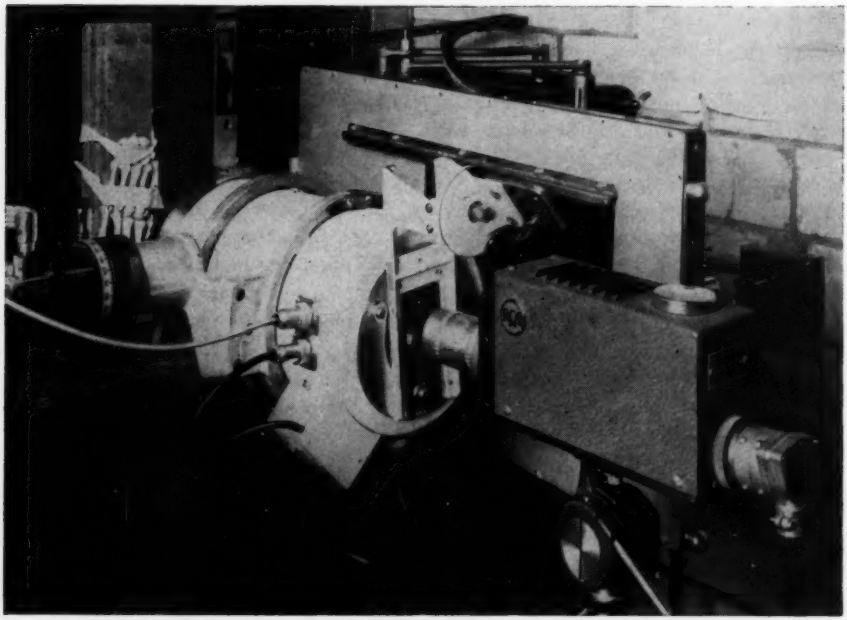
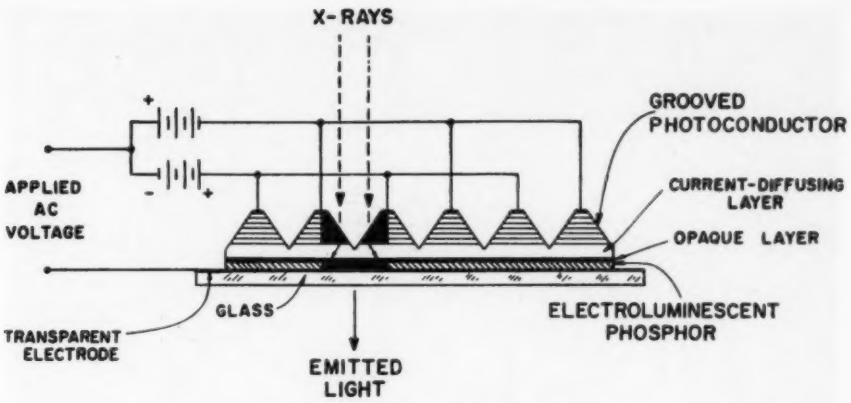


Figure 7
Fluoroscopy with Image Amplifier and Television Camera (Stauffer et el, reference 11).



Cross section of amplifying fluoroscope screen.

Figure 8
Cross Section of Amplifying Fluoroscope Screen (Kazan, reference 12).

ing it out and increasing alternating fields across the electroluminescent layer. The ridge construction of the photoconductor minimizes its electrical capacity.

Since both the photoconductor and the electroluminescent layer have highly non-linear electrical characteristics, the x-ray panel amplifier delivers pictures with high contrast. Brightness gains better than 100 have been achieved at low radiation levels.

However, before the x-ray panel amplifier can become a practical device replacing the conventional x-ray screen, improvements must be made in speed of response and in graininess of the image.

If x-rays occupy a leading position as a diagnostic technique, the microscope is beyond question the chief research tool of the biological scientist. Unfortunately, the organic preparations which form the main object of study are frequently so uniformly transparent at high magnification that discrimination of detail becomes very difficult. In the past, this difficulty has generally been overcome by the use of specific stains which combine chemically with different substances. This staining technique has the serious drawback however that it is generally lethal to living substance and may also cause structural changes. At the same time it is well known that substances which appear quite transparent in the visible commonly exhibit strong characteristic obsorptions in the ultra-violet portion of the spectrum. An example is provided by identical sections of kidney tissue observed under unltraviolet illumination with the wavelength of 2537 Angstroms and visible illumination of 4000 Angstroms. (Fig. 9). The cell nuclei stand out clearly in the ultraviolet picture.

The above pictures were obtained by observing the microspecimen with an ultraviolet-television camera and photographing the pictures observed on the screen of a

Figure 9

Identical Sections of Kidney Tissue, Photographed with Television Microscope with 4000 A and 2537 A Illumination, Respectively.

television monitor. Before the introduction of this technique the continuous observation at high magnification of objects illuminated by an ultraviolet source was impossible. Now, the Ultrascope has further simplified direct ultraviolet microscopy.

The Ultrascope consists essentially of an image tube (Fig. 10) and magnifier which, in an ultraviolet miscroscope, replaces the eyepiece of the conventional instrument. The image tube converts the ultraviolet image projected on its photocathode into a greenish-yellow image of high brightness on its fluorescent screen. Thus, with this electronic device, microscopy in the ultraviolet becomes as simple as in the visible range of the spectrum.

The picture observed with Ultrascope is, of course, in monochrome. Thus, while it does away with the necessity of staining with possible adverse effects on the specimen structure, it does not permit the immediate differentiation of various components which might be achieved with a complex multiple staining technique. Differentiation of color, without the drawbacks of staining, can, however, be achieved by color translation, employing highly developed color television techniques. In color translation, each of the three primary colors, red, blue, and green, is correlated with one of three selected ultraviolet wavelengths, for which the components of the specimen exhibit material differences in absorption.[13] Ultraviolet images of the specimen formed with

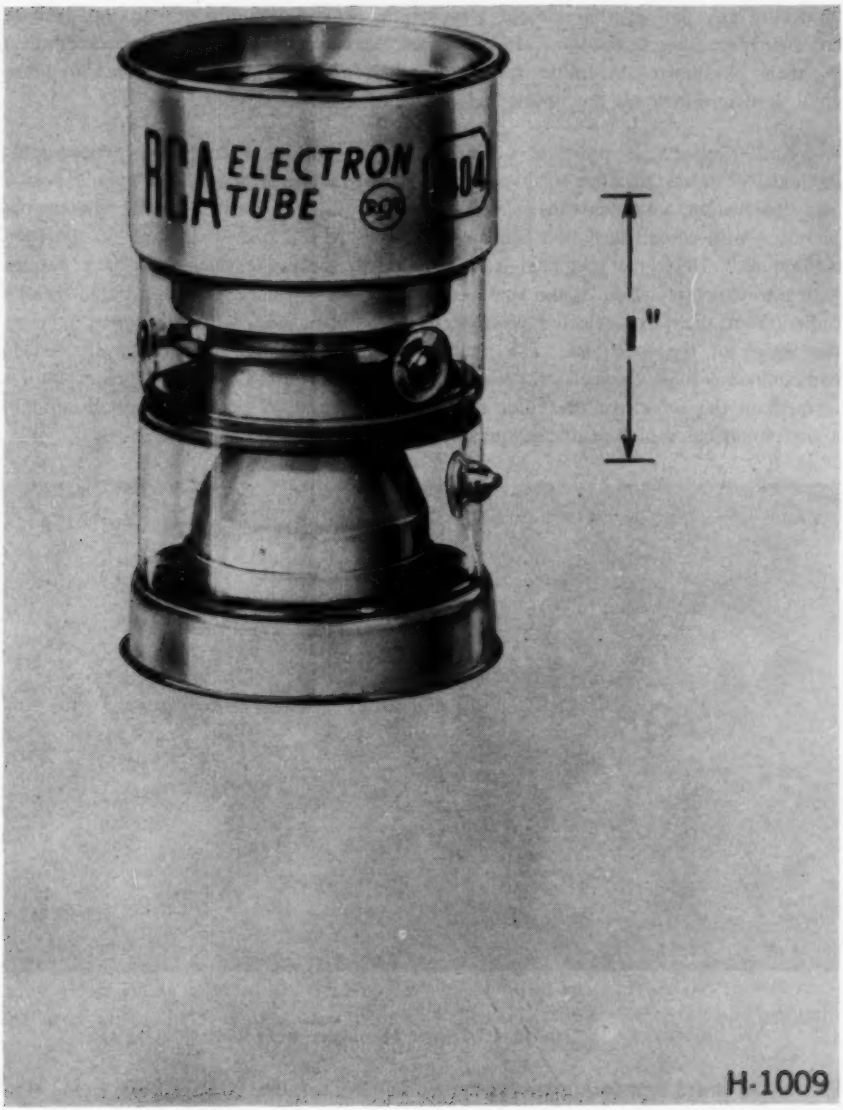


Figure 10
Ultrascope Image Tube.

these three wavelengths are converted into red, blue, and green images, which are viewed in superposition. Thus, cyan, yellow, and magenta portions of the compound image immediately indicate the presence of substances which strongly obsorb the ultraviolet radiations correlated with red, blue, and green, respectively. Substances

absorbing any two of the selected wavelengths will appear tinted with the primary color corresponding to the third. In brief, the color of different substances is determined by their ultraviolet absorption spectrum, much as in conventional microscopy the color is determined by the absorption spectrum in the visible.

Earlier efforts at practical ultraviolet color translation employed photographic methods.[14,15] They necessarily involved long time delays between specimen exposure and observation, and demanded large expenditures for materials. Instantaneous observation with color translation become possible only with the application of television techniques.[16] Here, the specimen is illuminated in successive vertical fly-back periods with ultraviolet radiation of the three selected vertical fly-back periods with ultraviolet radiation of the three selected wavelengths. An ultraviolet-sensitive camera receives the image of the specimen. The picture signals are channeled successively to the corresponding color channels of a television monitor. The red, blue, and green pictures formed on the screen of the color kinescope are fused by persistence of vision into a compound color picture of the specimen.

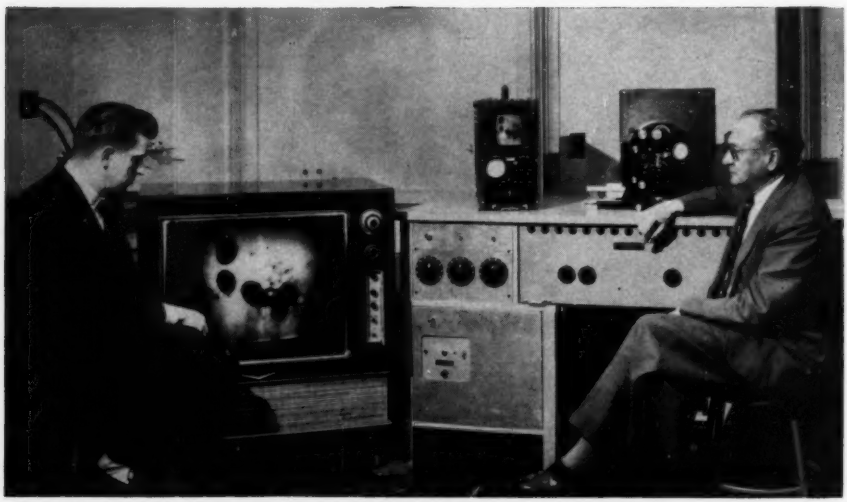


Figure 11

The Ultraviolet Color Translating Television Microscope at the Rockefeller Institute.

In the instrument constructed at the Rockefeller Institute in New York,[17] (Fig. 11) illumination is provided by three separate ultraviolet monochromators provided with thyratron-controlled mercury-arc sources. The radiation from the exit slits of the monochromators is directed into the substage of the ultraviolet microscope by a rotating 45-degree mirror. (Fig. 12). The striking of the mercury arcs, the vertical deflection of the television systems, and the gating of the three color channels are all controlled by commutator contacts on the mirror mounting, which has total rotation period of 1/20 second. A high-sensitivity image orthicon with an ultraviolet-trans-

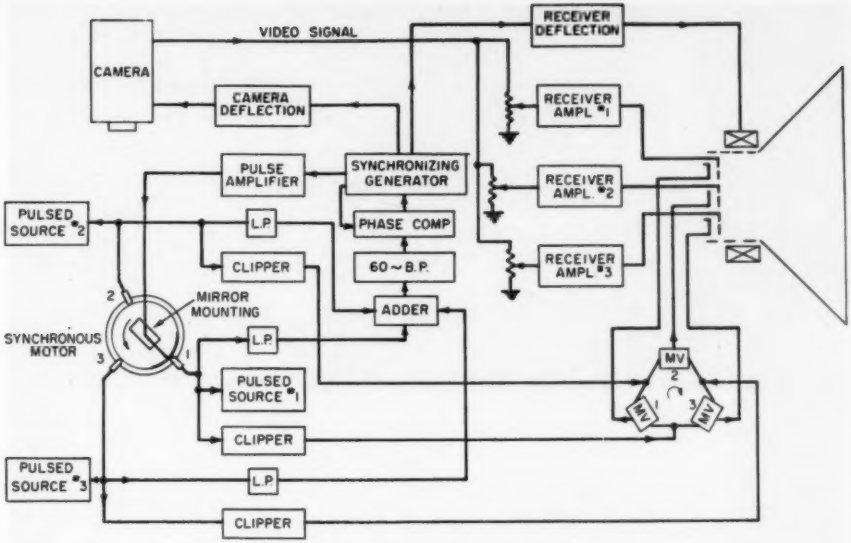


Figure 12
Block Diagram of Color Translating Microscope at Rockefeller Institute.

missive front end serves as camera tube. The monitor is a conventional color television receiver converted to field-sequential operation.

A good example of the type of picture provided by the ultraviolet color translating microscope is given by a specimen of nucleated red cells of frog blood. (Fig. 13). Here a wavelength of 315 millimicrons was correlated with red, 265 millimicrons with green, and 400 millimocrons with blue. The red portions of the picture correspond to the nuclei of the cells, where the wavelengths corresponding to green and blue are both strongly absorbed by nucleic acid. The hemoglobin in the body of the cell is responsible for the yellow tint, resulting from the absorption of blue. The white corpuscles appear magenta as the result ot the absorption of the wavelength correlated with green. Finally, a quantitative idea of the relative absorption of the different cell constituents at the selected wavelengths may be obtained by applying the signals from the three color channels to a line selector oscilloscope. This gives a visible representation of the variation in signal amplitude along a particular scanning line.

The primary aim of the various forms of ultraviolet microscopy which have been described is the accentuation of contrast, in the form either of brightness differences or of color differences, between different specimen components. The increased resolution obtainable, in view of the somewhat shorter wavelengths of ultraviolet as compared with visible radiation, is generally a secondary consideration. Whenever very high resolution is required in biological investigations, another electronic instrument fills the gap, the electron microscope. (Fig. 14). While this instrument has, for a long time, been the object of independent development, it too, in its early stages,

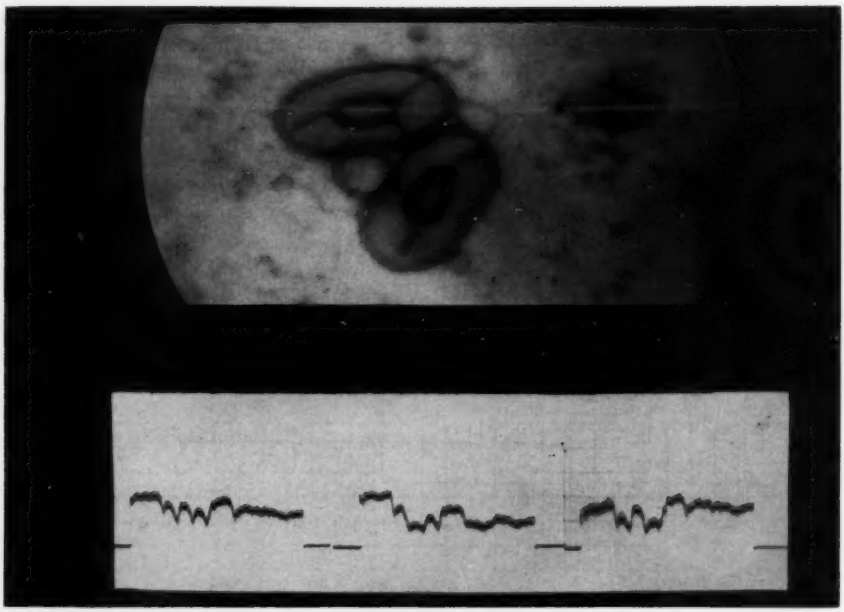


Figure 13

Picture of Nucleated Frog Blood Red Cell with Oscillograph Traces Indicating Absorption at 315, 265, and 400 millimicrons.

represented an application of techniques familiar from cathode-ray oscilloscope and television studies. The staff of the Edsel B. Ford Institute has contributed materially to the application of this powerful electronic research tool and is without doubt well aware of its potentialities.

In all of the problems considered so far the application of electronics has led to material progress. There are at least as many in which electronics may well provide the ultimate solution, although success has eluded us so far. An example is the measurement of intraocular pressure in a manner not painful to the patient. This is of great importance in the early detection of glaucoma, a blinding disease endemic in an aging population. Attempts to measure the pressure change optically or by a resulting change in the propagation time of ultrasonic pulses through the eyeball or along its surface are under investigation, but have not led to success. There is ample scope for new ideas and techniques for the solution of problems of this kind.

There are, finally, a number of ways in which the electronic engineer can contribute materially to the overall economy and efficiency of our health care. In particular, present practices of hospital operation suggest many possible points of improvement with respect to optimum utilization of skilled personnel and maximum economy. Thus, the replacement of the conventional thermometer with high-speed thermistor-probe could greatly accelerate the time-consuming process of temperature recording. An experimental thermometer of this kind, arranged for printing out the temperature,

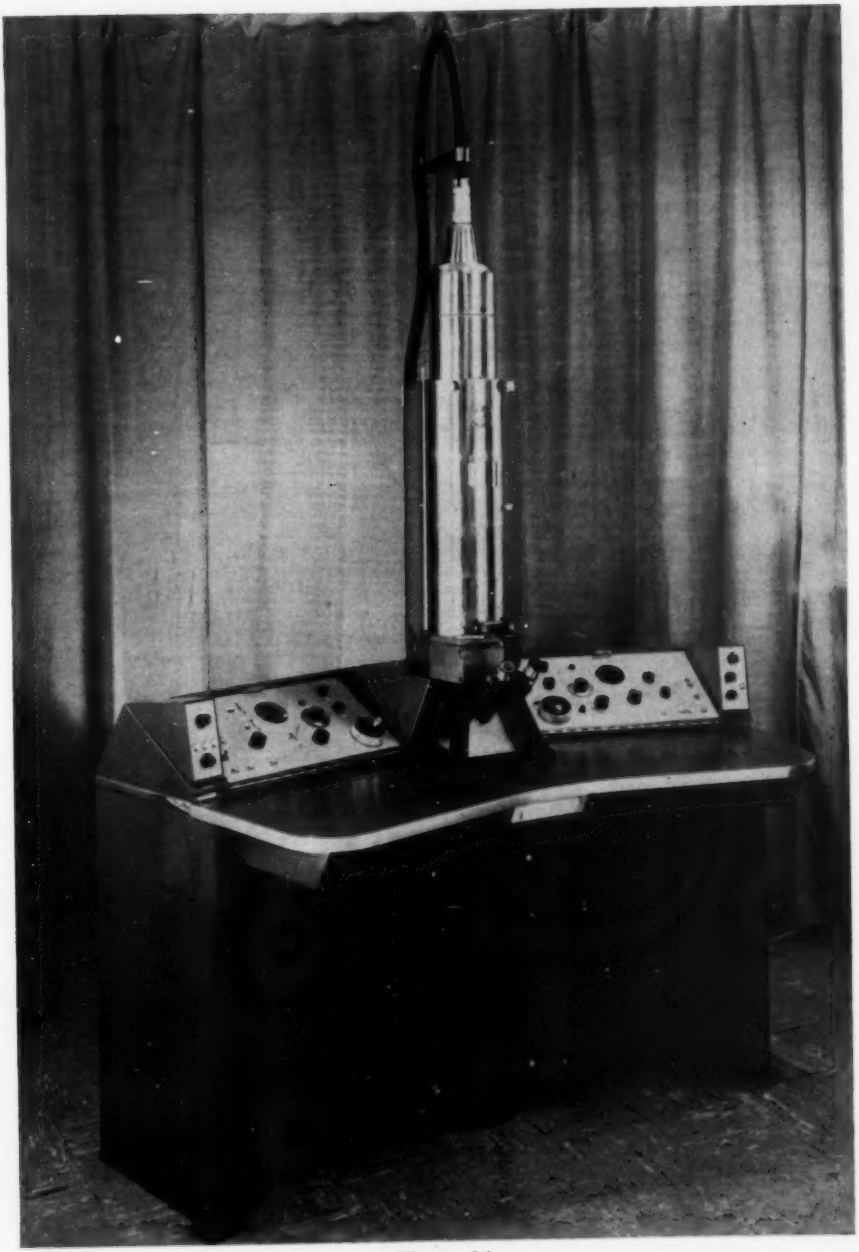


Figure 14
RCA EMU-3 Electron Microscope.

is shown in Fig. 15. In fact, with the development of suitable automatic recording equipment the skilled nursing staff could be relieved altogether of the routine of taking the patient's temperature and pulse, which could be taken over by less skilled personnel. Furthermore, automatic data transfer from the punched-card record of the recording instrument to the patient's record would both save time and eliminate error. Numerous other techniques could, without doubt, be borrowed from automation techniques currently applied in industrial practice to reduce the cost and improve the quality of hospital services.

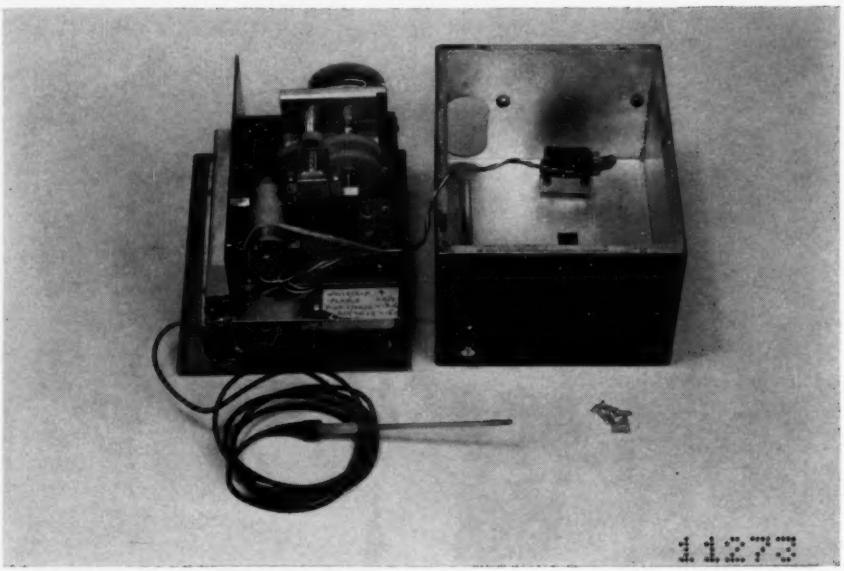


Figure 15
Experimental High-Speed Thermometer for the Automatic Recording of Patients' Temperatures.

The rapid increase in accumulated medical knowledge confronts us with an even more urgent problem. To an ever-increasing degree, individual physicians can not store in their memories the vast amount of information which has bearing on the proper diagnosis of disease from the symptoms exhibited by a particular patient. What he needs is the possibility of recourse to a much larger memory containing the countless correlations between symptoms, disease, and therapy, which are now scattered through the medical literature and the hospital records of the world. Modern computer technology has provided us with the vast, high-speed access memories needed for such a purpose and our communication system would permit such a single memory store to serve physicians scattered over a wide area. What is still lacking is the extensive research required to determine the best method of coding the available information in computer language and in planning the computer logic so that it may provide the needed answers.

A small beginning in this direction has been made. A group of physicians at the New York and Mt. Sinai Hospitals, in cooperation with the Medical Electronics Center of the Rockefeller Institute and the RCA Electronic Data Processing Division, have set up an experimental program for the differential diagnosis of the hematological diseases, making use of available hospital records. Ninety-eight different symptoms were employed as indicators for some 30 diseases. To obtain a diagnosis for a given hospital case, the observed symptoms were recorded on a tape and compared by the computer with the symptom listings for the several diseases, also recorded on a magnetic tape. On the basis of this comparison, the computer printed out a list of diseases consistent with the observed symptoms, as well as those untested symptoms which might facilitate a positive diagnosis. It was found that, in the great majority of test cases, a positive diagnosis or a relatively short list of possible alternative diseases was provided. It is expected that much further work will be done to refine and extend the procedure.

Diagnosis is only one important application of electronic data processing in the field of medicine. Other important services which it may provide are the maintenance of standardized health records for the population, the charting of the origin and the course of epidemics, and the analysis of statistical data on the effect of new drugs and health measures. The rapid access to needed information provided by modern electronic computer techniques is equally significant for the extension and for the rapid application of medical knowledge.

In summary, medical electronics offers a great array of novel techniques which can contribute materially to the fruitfulness of medical research and the effectiveness of medical practice. Their development and elaboration demands close cooperation of medical men and life scientists with engineers and physical scientists for the common goal of improving health and overcoming suffering. Such cooperation is realized to an increasing degree today. It will bear its best fruits when the public support given to this effort is commensurate with its importance for our public welfare.

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EVALUATION OF NEEDLE BIOPSY OF THE LIVER: 1950-1958

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Even though the technique of needle biopsy of the liver was reported as early as 1895, the first impetus for its use in this country came from the report of Iversen and Roholm in Sweden in 1939.[1] The early reports on the use of needle biopsy of the liver in the United States were by Hoffbauer[2] and Volwiler and Jones[3] in 1947. All of Hoffbauer's cases consisted of patients with hepatomegaly. On the other hand, 68 of 234 biopsies reported by Volwiler and Jones were through the transthoracic approach and in patients with apparently normal-sized livers. In these early reports, needle biopsy of the liver was recognized as an aid in understanding the pathogenesis of disordered hepatic function and for guidance in therapy.

Four major indications for needle biopsy of the liver were outlined in an earlier report.[4]

1. To define the stage of known liver disease for treatment and prognosis.
2. To diagnose certain hepatic pathologic conditions in which examination of the tissue is indispensable.
3. To disclose the presence of systemic disease in the liver.
4. To investigate and understand the pathogenesis of clinical liver disease.

The following case reports have been selected as examples to illustrate the value of needle biopsy under these criteria.

I. To define the stage of known liver disease.

CASE 1.

A 16 year old white female was referred by her local physician because of progressive jaundice of four months' duration thought to be due to viral hepatitis. The patient had been hospitalized three times before coming to Detroit. Her only complaint was malaise. There was considerable tenderness over the liver, which was enlarged three centimeters below the right costal margin. The serum iron was 178 mcg. percent. The liver flocculations were 4 plus. The zinc sulfate turbidity was 40 units. The total bilirubin was 4.2 mg. percent with a direct of 1.7 mg. SGO-transaminase was 546 units and decreased to 34 units after cortisone therapy was started. One month following admission a needle biopsy of the liver was done and mild to moderate periportal infiltrates were seen. The hepatic wedge pressure was elevated. Several L. E. tests were negative. The roentgen-study of the esophagus and stomach was normal.

Comment: During the following two years, the patient was followed with serum bilirubin determinations and steroid therapy was decreased gradually. Icterus increased when the steroid therapy was withdrawn. She was considered to be developing a post-necrotic cirrhosis with hyperglobulinemia and hypoalbuminemia. Heavy fibrous tissue in the portal areas was seen in a second needle biopsy done eighteen months after the first admission.

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CASE 2.

A ten year old boy was admitted with a three year history of Legg-Calvé-Perthes disease (osteochondritis deformans juvenilis) involving the left hip. The patient had been placed in a body cast for one month. One year later he was hospitalized on two occasions with a diagnosis of infectious hepatitis. The patient began to tire easily and had occasional epistaxis during the following two years. There were spider angiomata over the upper extremities and the anterior chest. No lymphadenopathy was found. The left lobe of the liver was enlarged, hard, and non-tender. The spleen extended 10 cm. below the left costal margin.

Laboratory studies included a white blood cell count of 4,150, serum bilirubin 0.65 mg., reticulocyte count 1.6 percent, albumin 3.3 gm., globulin 3.7 gm., cephalin cholesterol 3 plus, thymol turbidity 16 units, thymol flocculation 2 plus, bromsulfalein 17 percent and serum cholesterol 149 mg. percent. There was no bilirubinuria. The bone marrow was compatible with lupus erythematosus, but was not diagnostic. Two peripheral L. E. tests were suggestive but non-conclusive. A peripheral blood smear was normal. X-ray of the gallbladder was normal.

One month later the patient was re-admitted with severe left hip pain of 12 hours duration, pharyngeal injection and a temperature of 101 degrees. Hepatosplenomegaly was present. There was marked spasm of the left lower extremity with difficulty in movement of the hip. The leukocyte count was 8,500. The patient improved gradually and was discharged with a diagnosis of probable rheumatoid arthritis of the hip. During the following year the patient was observed as an out-patient. He gained weight and the liver and spleen were smaller in size. A few spider angiomata were present over the chest wall. The total proteins were 6.7 gm.; the white blood count was 3,550; bromsulfalein was 15 percent. The cephalin cholesterol was 4 plus, thymol turbidity was 6 units, and the thymol flocculation was 4 plus. Esophageal varices were seen in the x-ray of the esophagus. A liver biopsy was done and widespread fibrosis and lymphocytic infiltration were seen in the portal spaces. Re-duplicated bile ducts were prominent.

Comment: The stage of liver disease in this 10 year old boy was clarified by needle biopsy. The final diagnosis was post-necrotic cirrhosis.

II. To diagnose certain hepatic pathologic conditions in which examination of the tissue is indispensable.

CASE 3.

A 59 year old white male was admitted with a diagnosis of diabetes mellitus. There was a past history of peptic ulcer disease and mild icterus which was due to cholelithiasis. The patient's symptoms improved after dietary fat restriction. One year later the patient was re-admitted because of gradual weight loss. He appeared well-nourished but the skin had a sallow tint. The body hair was sparse and the skin was dry, atrophic and dusky. The chest was emphysematous. The lungs were clear. No cardiomegaly was present. The liver was palpable two centimeters below the right costal margin and was non-tender. The testicles were atrophied.

The complete blood count was normal. The fasting blood sugar was 283 mg. The urine had 2 plus glycosuria. The 24 hour urine sugar was 23 gm. Serum calcium

was 10.4 mg., serum phosphorus 3.6 mg. and the bromsulfalein was 5 percent. Free acid was present in the stomach. Kidney function was good. X-rays of the esophagus, stomach, duodenum, gallbladder and chest were normal. The kidneys appeared normal in intravenous pyelograms. The diabetes was controlled with protamine zinc insulin 20 units and an 1800 calorie diabetic diet.

The following year the patient was admitted with ankle edema, dyspnea, and fatigue. There were congestive changes in the chest. The heart was enlarged to the left. P^2 was greater than A^2. The liver was palpable 5 cm. below the right costal margin. Moderate pedal edema was present. The skin was pigmented over the exposed surfaces and had a brownish-gray cast. Right heart failure was thought to be due either to a myocardial infarct or to pericardial effusion. The venous pressure was 88 mm. of water and the decholin circulation time was 32 seconds. Flattening of the T waves was seen in the electrocardiogram indicating coronary atherosclerosis. There was fluid present in the right pleural cavity and left ventricular hypertrophy. The fasting blood sugar was 84 mg., the non-protein nitrogen 48 mg. and zinc sulfate 10 units. The serum bilirubin and liver flocculations tests were normal. The bromsulfalein test was 7 percent. The complete blood count was normal. Following slow digitalization the patient lost 10 pounds during a ten-day period. Liver biopsy at this time was diagnostic of hemochromatosis. Two venosections were done at monthly intervals following discharge from the hospital. Three months later the patient expired and at necropsy was found to have hemochromatosis involving the skin, liver, pancreas, myocardium, stomach and adrenals.

Comment: This disease was unsuspected initially. Tissue diagnosis is indispensable in hemochromatosis.

CASE 4.

A 42 year old housewife was admitted with a history of good health all of her life. She complained of fullness in the abdomen and pain in the right side radiating to the inguinal area. This discomfort was increased by sneezing and coughing. The present history began one month previously when she had developed an upper respiratory infection associated with generalized aching, fever, chills and cough. Because of prolonged asthenia following this illness, the patient was admitted to a local hospital where hepatic disease was suspected. Regenerating liver tissue had been seen in a liver biopsy.

At the time of her admission to Ford Hospital there was neck vein distention in the recumbent position. Spider angiomata were seen over the right shoulder and chest. No icterus was present. The breath sounds and resonance were decreased over the right lung base. The blood pressure was 120/80. A slight systolic apical murmur was present with a protodiastolic gallop. The liver was enlarged 4-5 cm. below the right costal margin and was not nodular.

The hemoglobin was 12.2 gm. and a leukocyte count of 12,650. The urine, non-protein nitrogen, and fasting blood sugar were normal. The liver flocculation tests were normal. The total proteins were 8.3 gm. with an albumin of 4.6 mg. The prothrombin time was normal. The stools for parasites were negative. The bromsul-

falein test was markedly abnormal (54 percent). Urea clearance indicated renal impairment (35 standard clearance). The Bence-Jones protein was negative. Cyroglobulins were negative. The L.E. test was negative. Diffuse amyloidosis was seen in a liver biopsy and a bone marrow biopsy was confirmatory of amyloidosis.

Comment: An unsuspected lesion was found in the liver biopsy of this patient in whom tissue diagnosis was indispensable to identify the hepatic pathology.

III. To disclose the presence of systemic disease.

CASE 5.

A 65 year old negro male was referred from another hospital. He had back pain of four months duration, anemia, and a 50 pound weight loss. The low back pain was non-radiating and interferred with walking and sleeping. The patient had been hospitalized four weeks previously. He was emaciated. There was moderate tenderness over the lumbar spine. Grade III arteriosclerotic changes were present in the eyegrounds. The heart was enlarged to the left. A grade II apical systolic murmur was present. The liver was enlarged and the prostate also was enlarged with a nodule in the left lobe. There were nucleated red blood cells and young myeloblasts in the peripheral blood. The spine had osteoblastic changes. Acid phosphatase was 4.5 mg. A prostate biopsy was negative for pathology. Agnogenic myeloid metaplasia was diagnosed by needle biopsy of the liver.

CASE 6.

A 31 year old male Arab was admitted because of the acute onset of headache, arthralgias in the hands, elbows, shoulders, neck, right hip, knees and ankles. He had been vomiting and had developed diarrhea with a fever of 102 degrees. Schistosomiasis mansoni ova were identified in the stool examination. This infestation was found also in the biliary drainage. The patient stated that previously he had been swimming in contaminated water in his native country of Yemen. The bromsulfalein test was 6 percent. The hemoglobin and leukocyte count were normal. A Schistosome ovum was found in the liver biopsy with marked tissue reaction around the ovum.

IV. To investigate and understand the pathogenesis of clinical liver disease.

CASE 7.

A 47 year old negro male was admitted with a right lobar pneumonia. Sputum cultures with sensitivity studies were done and a coagulase positive staphylococcus was found with sensitivity to penicillin and streptomycin. The patient's course after two weeks was unimproved. Novobiocin was added to the antibiotic therapy. Ten days later the patient's temperature returned to normal but he was noted to be icteric and obstructive jaundice was present. A transduodenal drainage was normal with no evidence of biliary calculi. During the subsequent four months the jaundice cleared and the patient was improved. At this time bile stasis was seen in the needle biopsy of the liver. Six months later a repeat liver biopsy was interpreted as normal. The patient has remained well.

Comment: This patient represents an obstructive jaundice concomittant with Novobiocin therapy recently reported by Haubrich.[5]

The development of the hepatic changes were demonstrated by needle biopsy. Histologic evidence that the liver had healed was secured by a second needle biopsy of the liver after a period of convalescence.

COMMENT

From 1950 through 1958 there were 741 biopsies performed. Of these 64 percent confirmed the clinical impression, 9 percent corrected an erroneous clinical diagnosis, and an unsuspected lesion was found in 6 percent. Needle biopsy of the liver was helpful in establishing the diagnosis in 79 percent of the patients studied. Biopsy findings were non-specific in 13 percent, neither confirming nor correcting the clinical diagnosis. Misleading biopsy findings were found in 5.5 percent. An unsuccessful biopsy or inadequate specimen was obtained in eighteen patients (2.5 percent).

Gamble and Sullivan[6] have reported 81 percent diagnostic value in 323 needle biopsies. Ward et al[7] found that needle biopsy indicated the proper diagnosis in 74 percent of 1114 biopsies on 1000 patients. Fisher and Faloon[8] have reported the biopsy to be not consistent with the initial clinical diagnosis in 30.2 percent. We have found the number of unsuspected lesions discovered by needle biopsy to have increased since an earlier report.[4] Hemochromatosis has been found in several patients in whom the clinical diagnosis had been suspected of cirrhosis, nephrosis and amyloidosis.

Table I

Pathologic Diagnoses	No. Cases	
Portal Cirrhosis	294	(11)*
Normal Liver	108	(4)
Malignancy	65	
Metastatic carcinoma, hepatoma, lymphoma		
Hepatitis	52	(6)
Fatty Liver	42	(2)
Bile Stasis	41	
Hemochromatosis	26	(9)
Inadequate Specimen	18	
Biliary Cirrhosis	11	
Sarcoid	7	(1)
Chronic Passive Congestion	6	
Miscellaneous	38	
Including granulomata, miliary tuberculosis, glycogen storage disease, agnogenic myeloid metaplasia, postnecrotic cirrhosis, amyloid, polyarteritis, Schistosomiasis, et al.		
	708	(33)*

*Second biopsies, duplicate diagnoses.

In Table 1 are tabulated the pathologic diagnoses from 741 biopsies. The incidence of various anatomic lesions has remained about the same since 1954.[4] The diagnosis of normal liver tissue in 112 biopsies is attributed to the use of this diagnostic technique in various systemic disorders which occasionally may involve the liver. These have included lupus erythematosus, subacute bacterial endocarditis, polyarteritis, 'cardiac' cirrhosis in chronic congestive failure, metastatic malignancy in the liver and attempts to diagnose early hemochromatosis in patients with diabetes mellitus. Approximately two-thirds of the 294 patients with portal cirrhosis have been classified as mild or moderate.

Needle biopsy of the liver has continued to be an important technique in 'looking for' metastatic disease. Needle biopsy was diagnostic of metastatic carcinoma in 53 patients (74 percent) of 71 patients in the series who were proven to have carcinoma in the liver. Subsequent laparotomy or necropsy in 18 patients proved that there had been no metastases to the liver. Hence the diagnostic accuracy in 'looking for' metastatic disease in the liver was 79 percent. Eight of twelve lymphoma's were found by liver biopsy. Although two or three penetrations might have been made at the time of the initial biopsy, the percentage of accuracy reported includes only a single biopsy in each patient. An increase in the diagnostic accuracy in finding metastases is to be expected if a second biopsy is done after a progress interval of time.[9]

Of the patients biopsied in this series, 84 percent had hepatomegaly, 28 percent were icteric, 18 percent had splenomegaly and 9 percent had ascites. This diagnostic test is done with its specific indications balanced against the possible risk. We have had one death (0.13 percent mortality) from bile peritonitis in a geriatric patient with obstructive jaundice. Back pressure of bile or blood in the liver is a precise contraindication for the procedure. Purulent infection in the area of approach to the liver is also an absolute contraindication. In one patient with systemic actinomycosis the biopsy needle entered a liver abscess without serious sequelae. Relative contraindications include the uncooperative patient, bleeding tendencies, impaired blood coagulation and ascites. Occasionally patients have pleural pain following the procedure and require intercostal block anesthesia. We have had one patient develop pneumothorax after the biopsy procedure and three patients had a mild subcutaneous emphysema. The intercostal approach was used in 702 biopsies and the anterior subcostal approach in 39.

SUMMARY

The value of needle biopsy of the liver has been evaluated from an experience of nine years. The technique has aided in a proper diagnosis in 79 percent of the patients studied. Case reports have illustrated the precise indications and value of the procedure.

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PRESENCE OF MICROSCOPIC CRACKS IN VIVO IN BONE

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Some years ago while the writer was demonstrating the peculiar localization of cracks in ground sections to Dr. Charles O. Bechtol, the latter raised the intriguing possibility that the cracks need not necessarily be all artifact. This and Dr. Bechtol's engineering background provided the stimulus for the investigative work leading to this paper, which is the first of three dealing with the subject.

In the engineer's vocabulary the word fatigue has a special meaning: weakening of a strucutral member under repeated loads leading to failure. The faliure begins as a series of minute cracks which gradually extend in depth and length until the remaining intact material is insufficient to withstand a single load. The study of fatigue is complicated by many factors, among them being surface defects of form and composition, corrosion, the loading, the incompleteness of stress reversal during the loading cycle, and the composition, homogeneity, grain and phase structure of the structural material. The fatigue process is still not well understood, even in extensively studied metals[2,6]

The definition of fatigue suggests to an orthopaedist that a similar factor might be at work in the human skeleton. If so, one of its manifestations should be the existence of microscopic cracks in the bones. The problem then resolves itself into a two-fold one: unequivocal demonstration of such cracks as an in vivo phenomenon, followed by study of the cracks (if found and if we can be sure they are not artifact) in sufficient numbers of patients, pathological states, bones and experimental situations to assign useful meaning to them for academic and clinical purposes.

Unequivocal demonstration of in vivo cracks appears to be a simple project but did not prove so simple in execution. First it was necessary to stain such cracks, and second to visualize them on a cross section of bone in such a manner that artifacts could either be prevented or be readily distinguished from in vivo cracks. The writer believes that in vivo cracks have been demonstrated conclusively and this paper will present the evidence for this belief, leaving to subsequent publication the correlation of the cracks with stress, age, disease and some of the peculiarities of bone mineral elaborated in his laboratory.

MATERIALS AND METHODS

The *Materials* consist of 14 ribs from 14 patients, 10 males and 4 females. The ribs were resected during a thoracotomy in 10 cases and removed at postmortem in 4 cases. The ages of the patients ranged from 18 to 82 years. Because of the possibility that the periosteal elevator used in surgery might produce cracks during periosteal stripping the ribs removed at postmortem were resected with the scalpel.

The *Methods* finally adopted are as follows:

(a) A length of rib 3" or more was immersed in 1% basic fuchsin in a 40% ethyl alcohol solution and allowed to stain therein for 4 weeks. Nearly complete staining of all the physiologic spaces and cracks in the bone results. Staining is done at room temperature and the volume of the solution should exceed the volume of

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the specimen by a factor of ten. Basic fuchsin is so poorly soluble in pure water that unalcoholic solutions are very ineffective bone staining reagents and in addition are plagued by a peculiar failure to penetrate the bone's physiologic spaces. Drying in vacuo prior to staining, staining at elevated pressures or temperatures and staining in 95% or absolute alcohol were rejected at the beginning of this project because of the possibility that they might introduce artifacts.

An essential part of this technique is that the bone specimen to be stained be as fresh as possible when immersed in the stain solution. Prior fixation is not necessary since the alcohol in the staining solution produces what fixation is needed. Dehydration or defatting in any solvent prior to staining introduces the possibility of artifact due to differential shrinkage effects.

(b) At the end of the staining period the bone specimen is transferred from the staining solution to a large volume (exceeding the specimen volume by 100 X or more) of tap water and allowed to remain in this solution with one change to fresh solution for at least 48 hours. This step accomplishes several things. First, sections containing as little as 15% alcohol are unmanageably brittle when ground and will therefore fragment, seriously impairing the usefulness of the preparation. Thoroughly hydrated bone on the other hand may be ground extremely thin in complete cross section since it is then not only quite flexible but reasonably tough.

Second, basic fuchsin is poorly soluble in water but readily soluble in alcohol. If section grinding (the next step) is done with partly alcoholated bone some of the fuchsin will diffuse into the lubricating solution used while grinding and then be able to stain cracks which are artifact while grinding is in process. In thoroughly hydrated bone on the other hand only particulate stained debris may be forced into the exposed surface of artifactual cracks but these cracks will not be stained through the depth of the section. As a result with microscope objectives having numerical apertures exceeding 0.65, cracks stained through the depth of the section can be readily distinguished from those which merely have some particulate matter wedged into the surface but no stain in depth. Such artifacts occur frequently, especially in bone from older patients.

(c) After hydration, the bone specimen is then supported by hand in a wedge-shaped holder fashioned by placing 10 or more folded paper towels in the partly open jaws of a vise. Cross sections about 2 mm thick are sawed from the middle 1/3 of the specimen with a very fine-toothed saw using little pressure and rapid motion. The purpose is to cause as many teeth to perform the cutting action per second as possible. Failure to achieve this may cause the section to fragment during grinding. The section is then ground to a thickness of 30-50 u and mounted by techniques published elsewhere.[3] The grinding should be done under gently running water so that debris is flushed away. Otherwise the section surfaces become covered with adherent debris which interferes with microscopic observation of the section.

(d) From one to 5 complete cross sections per case were prepared and examined. In 10 of the cases longitudinal sections were also prepared to determine the length of the cracks and aid in their localization in the bone. In two of the cases fortuitous

preparation provided what in essence are serial sections, allowing for the thickness of the bone removed by grinding. Sections which were incomplete due to fragmentation in grinding were not included because of the possibility that the number of cracks might vary systematically in varying quadrants. This proved to be the case.

(e) To be accepted as a crack, the following criteria proved necessary in the light of experience.

First — the crack had to be stained through the depth of the section. Any crack not so stained was considered artifact in spite of the probability that some of them were in vivo cracks unstained due to poor penetration of stain.

Second — the crack had to open visibly onto the surface of the section. The air drying preceding mounting in resin usually (by virtue of a small amount of shrinkage occurring during drying plus variations in lamellar orientation and degrees of mineralization in different parts of the section) causes cracks opening into the section surface to spread apart slightly so that optically resolvable space exists in the crack.

Third — There had to be no permeation of the stain into the bony substance in the walls of the crack. Repeatedly the writer has observed, during the examination of routinely prepared ground sections, a zone of fuchsin permeability spreading outward from the surfaces of cracks caused both accidentally and deliberately (while evaluating the effect of various methods of section preparation on the permeability of fresh bone). This zone of fuchsin permeability varies from several to 50 u or more and for the present purpose must serve as indication of probable artifact.

(f) Quantitative determination of in vivo cracks is not attempted in this paper. Before we walk we must learn where to walk. Such determination would necessitate the following conditions and measurements: knowledge that every in vivo crack present can be stained; accurate counts of the number of cracks, total length and average length of cracks per unit area of cross section; homogeneity — or lack of it — of cracks per unit area at various levels in various bones plus correlations with cortical thickness, magnitude of physical stress and associated inhomogeneities of bone such as vascular porosity, feathering, micropetrosis, osteocyte death and proportions of Haversian to extrahaversian bone. It becomes evident that these are matters for future work and technical development.

(g) While observing the cracks which were present and which fulfilled the criteria listed under (e) it became evident that there were at least 4 types according to distribution. These types are cracks occurring in the plane of cement lines, cracks running parallel to lamellae and cracks crossing lamellae obliquely or perpendicularly. A much smaller group consists of cracks which invade Haversian systems. The counts of cracks are so tabulated in Chart I.

RESULTS

The information in Charts I and II will be summarized here, and in addition some interesting features of tentative significance will be described which are not presented in the Charts.

CASE	AGE	SEX	OR PM	COMMENT	SECTIONS EXAMINED	TOTAL NUMBER OF CRACKS SEEN			
						Parallel Lamellae	Perpendicular Lamellae	In Cement Line	In Haversian System
924416	18	M	OR	Dyspnea	2	1	1	23	1
924957	21	M	OR	Hiatus Hernia	3	1	2	3	0
914892	22	F	OR	Bronchiectasis	2	0	1	1	0
927890	23	F	OR	Patent Ductus	3	1	1	15	0
933423	26	M	PM	Acute Trauma	3	1	2	1	0
831088	28	M	OR	Hematemesis	2	0	11	5	0
459576	48	M	OR	Cough	5	16	39	33	1
856829	55	F	OR	Osteomalacia	3	4	3	7	0
928071	55	M	PM	Dyspnea	2	11	3	18	0
926196	57	M	OR	Asymptomatic	2	3	14	11	0
722345	58	F	OR	Hiatus Hernia	3	0	1	6	0
929122	58	M	OR	Ca. Esoph.	2	6	6	13	0
135720	61	M	PM	Cough	3	2	8	3	0
015364	82	M	PM	Myo-Infarct.	1	2	0	1	0

Chart I

Number and Placement of Cracks Seen
OR=Specimen from operating room
PM=Specimen obtained post mortem

CASE	CRACKS PER SECTION		
	Parallel	Perpendicular	Cement
924416	0.5	0.5	11.5
924957	0.3	0.6	1.0
914892	0.0	0.5	0.5
927890	0.3	0.3	5.0
933423	0.3	0.6	0.3
831088	0.0	5.5	2.5
459576	3.2	7.8	6.6
856829	1.3	1.0	2.3
928071	5.5	1.5	9.0
926196	1.5	7.0	5.5
722345	0.0	0.3	2.0
929122	3.0	3.0	6.5
135720	0.6	2.7	1.0
015364	2.0	0.0	1.0
Average for Group	1.1	2.2	3.9
Average for Group less than 30 years old	0.23	1.3	3.5
Average for Group more than 30 years old	1.7	2.9	4.2
12 Males	1.7	2.9	4.5
4 Females	0.4	0.5	2.4

Chart II

Number of Cracks per Section

(A) In all sections examined some cracks fulfilling the criteria in Methods, (e), were found. 36 complete cross sections were examined.

(B) The localization of the cracks in extrahaversian bone* is striking. Of a total of 280 acceptable cracks examined only 2 invaded Haversian systems, the remaining 278 ending rather abruptly at the cement line separating Haversian systems from extrahaversian bone.

(C) 50% of the acceptable cracks examined occurred in the plane of cement lines, while 33% were oriented in the cross section perpendicular to lamellae and 17% ran parallel to the lamellae. 1.8% crossed cement lines to continue in adjacent lamellae and only 0.7% entered Haversian bone as defined in footnote (*). (Slide rule accuracy only).

Figure 1A

50X, cross section 28 year man's rib stained and sectioned as described.

(D) Many planes, particularly in cement lines, were seen which stained in depth with fuchsin but which did not have a visible physical crack. These stained planes in cement lines occur within a micron of the outermost, optically distinguishable edge of the cement lines and are themselves only a micron or so thick. Another, less frequently observed phenomenon was the appearance in extrahaversian bone of somewhat similar plans of fuchsin staining which were quite wavy and fibrillated in

*Footnote: Extrahaversian bone is for the purpose of this paper defined as all bone in a section which is not intact Haversian systems. In so-called double and triple systems, only the inner system or ring is considered Haversian, the remainder being included in the extrahaversian moiety. Fragments of remodelled Haversian systems are also considered to be extrahaversian. This classification is not entirely arbitrary and is based on other work from the writer's laboratory wherein consistent differences in brittleness, hardness, osteocyte death, micropetrosis, feathering, permeability, susceptibility to etching in acid buffers and in shrinkage exist between the Haversian and extrahaversian moiety as defined herein. Previous definitions of extrahaversian bone (interstitial lamellae) lack sufficient precision for the present needs.

appearance but which also could not be shown to possess the physical discontinuity needed to define them as cracks. It is suspected that these are cracks in genesis. (Figure 4).

(E) In some sections careful examination would reveal cracks which, when followed down into the depths of the section, gradually or suddenly expanded, the space occurring having stained cells on the surfaces of the walls and in some instances a capillary vessel in the lumen. The walls of such spaces are often partly scalloped by Howships lacunae. (Figure 3),

(F) Attempts to correlate resorption spaces with the presence of cracks have so far failed to show a correlation, although such a correlation is anticipated by the writer.

(G) In cases 459576 and 831088 the final sections were ground from portions of the ribs sufficiently adjacent that continuity of trabecular and Haversian structures is evident when comparing the sections. In these sections the cracks which can be seen on one of them are found to continue in the same direction and moities of the extrahaversian bone in the section from the adjacent level. Such continuity is especially convincing when one has doubted, as the writer has, the conclusiveness o fhis findings. (Figures 1 and 2).

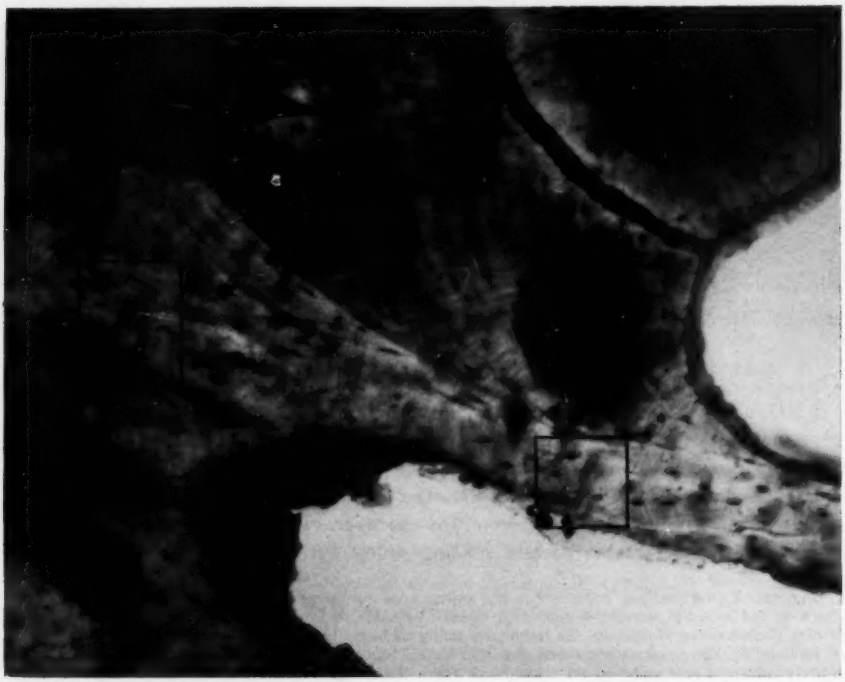

Figure 1B

Same as 1A, but higher power to reveal cracks (outlined running longitudinally in a moiety of extrahaversian bone.

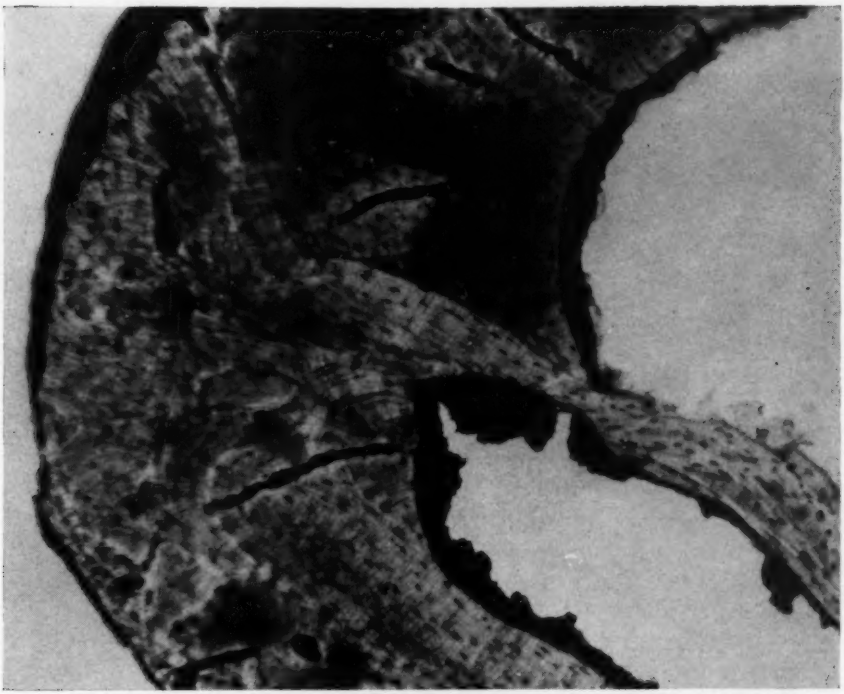

Figure 2A

Same specimen, 50X, section less than 1.0 mm distant from section in Fig. I. The trabeculum at right leads to a moiety of bone which is continuous with the moiety comprising the lower portion of the trabeculum seen in Fig. 1A.

(H) Examination of longitudinal sections prepared from the present material reveals that the cracks course in length to a variable degree ranging from 20 - 200 u and occasionally longer. On cross sections the cracks range in length from 10 - 100 u, occasional longer ones being seen in cement line planes between different portions of circumferential lamellae.

(I) The limited amount of available material suggests that cement line cracks occur with greater frequency in bones from young patients than in bones of older patients. Older patients on the other hand seem to develop more cracks in the extra-haversian bone than young ones.

(J) There does not appear to be any significant difference in the incidence of cracks between material obtained from the operating room and that removed at postmortem.

(K) Aritfactual cracks are found in most, but not all, of the 42 cross sections examined. By virtue of poor stain penetration some of them are probably in vivo cracks but cannot be included as such in the present work. Even in completely stained sections artifacts are usually evident and are unstained. Occasionally the

stresses produced during grinding lead to the extension of an in vivo crack. These are recognized by the stained in vivo portion and unstained artifactual portion.

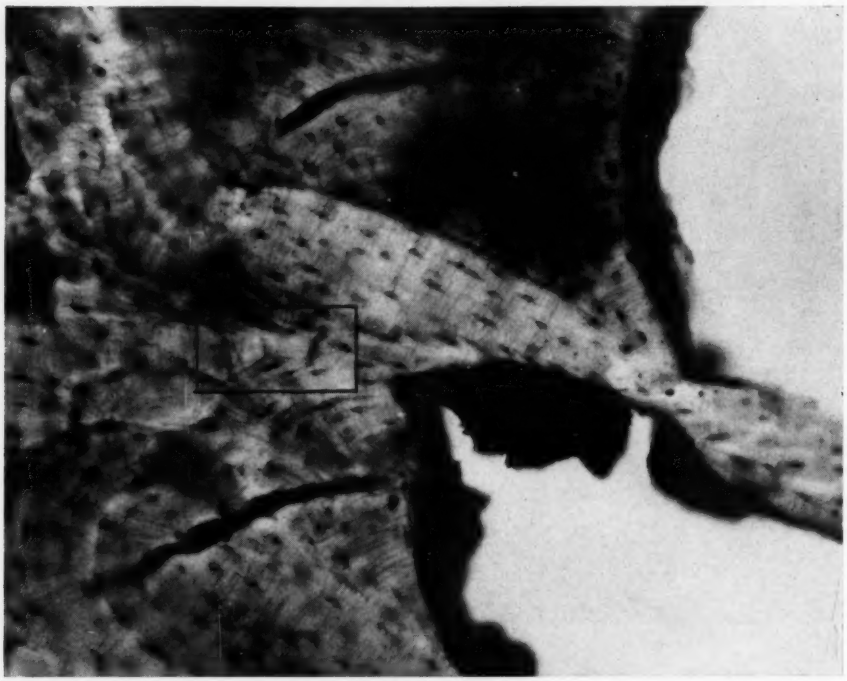

Figure 2B

Higher power of 2A. Three cracks are contained in the rectangle.

DISCUSSION

It is a moot point at present whether the cracks demonstrated by the writer are fatigue cracks or not. This must be proved by further work. The inference that they are the result of a fatigue mechanism is clear however. It will be of considerable interest to see if, as many suspect, these cracks play an important role in the production of some hip fractures, some spontaneous vertebral collapses in osteoporosis, the genesis of some cases of spondylolisthesis and the migration of some endoappliances attached to or partly embedded in bone. The writer has partial data available which suggest that this is the case.

If cyclic loading of living bone is the cause of the cracks, rather than some other less obvious factor, then the distribution of the cracks suggests that the affected parts of the bone are either weaker or subjected to larger stresses or both and it will be of interest to investigate and quantitate these factors.

The pronounced tendency of Haversian systems to be avoided by in vivo cracks and as previously reported by grinding cracks[4,5] leads one to the conclusion that they are either much stronger than extrahaversian bone or much more elastic. The first thesis cannot be supported in the light of available evidence. In addition to work from this

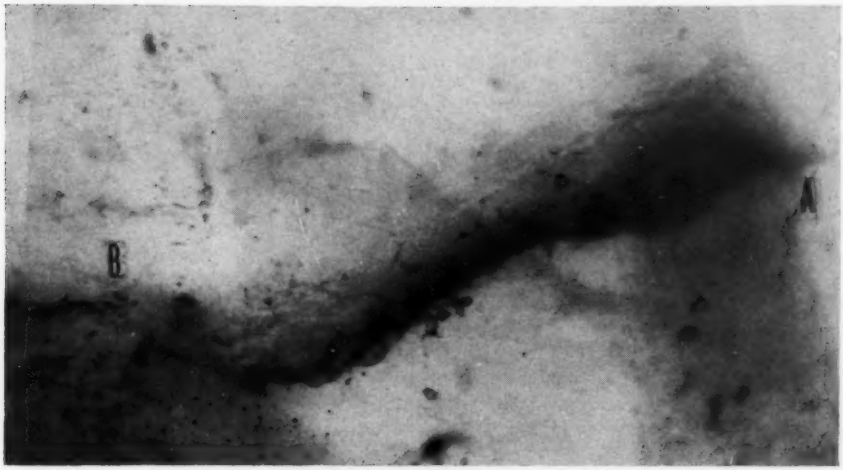

Figure 3A

Cross section 48 year man's rib. Focused at surface of section revealing serpentine winding of crack from the endosteal surface (Point A) to the edge of an osteon (at point B).

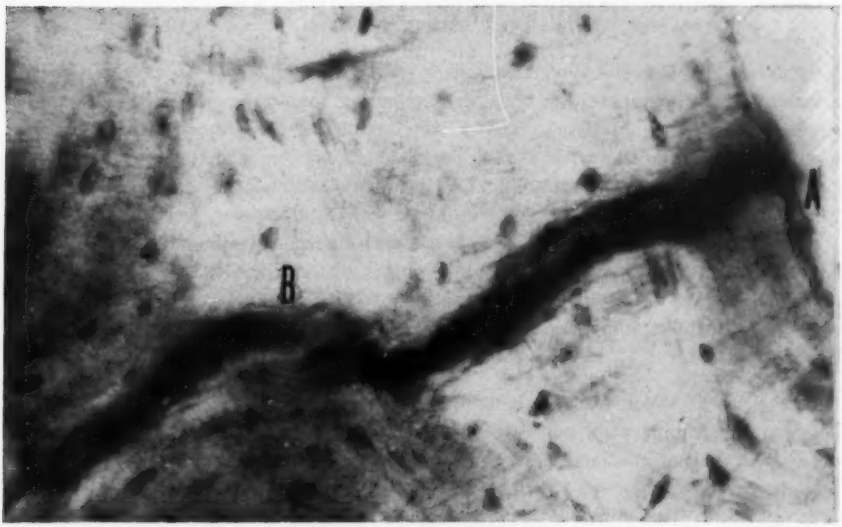

Figure 3B

Same field as 3A but less magnification, plus Wratten 25 filter. A vascular channel underlies the crack and contains a capillary vessel. Focused 45 μ under section surface.

Figure 4

Another field from same section as Fig. 3, the line labelled A is a crack stained in depth through the section. The line labelled B is a plane of Fuchsin staining which is not yet a crack. Both planes lie in the cement line separating the bone above from that below.

laboratory which indicates Haversian bone, particularly in older people, to be less densely mineralized than extrahaversian bone, most published microradiographic studies demonstrate the same thing. It is also known with reasonable certainty that the amount of matrix, once formed, does not change during mineralization and that the initial water content of the matrix is high but diminishes as mineralization density increases, the lowest water content (exclusive of that lying in bone's physiologic spaces) occurring where the greatest degree of mineralization exists. It is known that poorly mineralized bone in bulk is more easily strained than normally mineralized bone.[1] These facts, when assembled, make it awkward to assume that the sparing of Haversian systems from cracks is on any other basis than an increase in flexibility compared to the surrounding extrahaversian bone.

The cracks which lead down to a space containing some cells on the walls and occasionally a capillary are suggestive of a repair process. Certainly if the in vivo cracks occur by a fatigue mechanism they must either be repaired or accumulate, leading in the latter event to collapse of the entire skelton. With certain reservations we know this does not occur. Therefore, the cracks cannot be accepted as fatigue in nature unless a repair process which removes them is also observed. While the writer feels that the spaces observed and described with their occasional Howship's lacunae probably are crack repair processes, it is necessary to observe the end product and the transition

phases of the phenomenon before feeling can be substantiated. These observations have not yet been made.

The nature of the strains and other factors causing the cracks is of course speculative. However, their longitudinal orientation is consistent with the thesis that shearing strains are the predominant cause. The evidence supporting this thesis is: the known production of horizontal shearing strains under bending which are maximal in the plane of the neutral axis; the known production of longitudinal shearing strain in a structural member under torque;[6] the known weakness of cortical bone under longitudinal shearing strain as opposed to radial or tangential shearing strain;[1] and the known fact that torque and bending loads are the most frequent loads our long bones are subjected to.

SUMMARY

Three-inch or longer lengths of fresh, intact ribs obtained from the operating room and at postmortem were stained, sectioned and examined for in vivo microscopic cracks, utilizing simple techniques and procedures. Cracks which the writer interprets as existing in vivo were observed and exhibit a curious predilection for cement line planes and extrahaversian bone.

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SOME ASPECTS OF THE MECHANICS AND DYNAMICS OF BLOOD-BONE INTERCHANGE

H. M. FROST*

INTRODUCTION

This is an interpretive paper. Its first purpose is to outline some failings of the hydroxyapatite-hydration shell theory. Its second is to depict how other known facts of bone structure, physiology and physical chemistry can provide better understanding of blood-bone interchange phenomena when superimposed on the known features of the hydroxyapatite-hydration shell complex. Its third purpose is to inject new and necessary ideas into a field that is disorganizing because of lack of perspective.

This paper, then, is intended to be a stimulus rather than a reference.[1,17] Its documentation is accordingly general and selected for its provocativeness. Much of the information referred to is based on the writer's research.

Such a publication must be justified. Justification here is the increasingly widespread use of an incomplete theory to interpret data, particularly data derived from radioactive isotopes. Other factors than the hydroxyapatite crystal are ignored although they are more important in the sum total of observed interchange data than the hydroxyapatite-hydration shell complex alone. A broader perspective is needed.

ORIENTATION

Known facts will first be presented that point out the nature of the problems presently presented in blood-bone interchange studies.

1. *Bone Seeking Substances:* Since John Hunter we have known that alizarin deposits in newly forming bone and remains fixed there until remodelling processes release it by destroying the host bone. Many bone-seekers are now known and are the subject of much clinical, public health and scientific interest.

Some bone seekers are harmful in the body fluids and skeletal deposition is one means of decreasing their concentration in the plasma to safe levels (lead). Some are harmful in the skeleton and their removal and excretion would be preferred (radium, Sr^{90}). Some are helpful in one concentration and harmful in another (fluorine). Some become firmly fixed in the skeleton (radium, cesium), others loosely fixed so that various physiological circumstances may cause their reappearance in the blood (Yttrium, strontium, sodium). Some are adventitious and important only as means of studying bone in vivo (tetracyclines).[8,12]

In any case these substances behave in diverse and often diametrically opposed ways, in response to physicochemical and physiological factors which may be guessed at but are not at present known. The clinician would have a potent diagnostic and therapeutic weapon if he could cause various elements to deposit in the skeleton and leave it at will.

The hydroxyapatite theory alone cannot explain the diverse behavioral modes, rates and quantities of blood-bone interchange. In some quarters this is being recognized.[11,13]

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2 *Skeletal Buffering:* It has long been suspected that skeletal buffering of acidosis and alkalosis occurs. It has been proved in vivo in animals. Several theories of the chemical mechanism involved have been proposed. Nevertheless skeletal buffering in man has been ignored although an ability to manipulate the fundamental mechanisms involved would be another potent clinical weapon.

The skeleton contains the largest reserves of buffering materials in the body. There is evidence that in chronic acidosis the skeletal reserves are utilized. We need to know how and why so that the fundamental mechanisms may be manipulated in acute situations to the benefit of the patient.

3. *Hyperparathyroidism:* Much information about hyperparathyroidism has been accumulated. The role of the kidney is appreciated and further understanding of its role imminent. In the process of learning about the kidney the skeleton became lost and our knowledge of the skeletal dynamics of this disease is rudimentary, sophistries to the contrary notwithstanding. Evidence turned up in recent years leaves little doubt that the skeleton is as, or more, important than the kidney in this disease. Attempts to integrate the hydroxyapatite-hydration shell theory into the pathophysiology have been comic in outcome. This theory explains only a small part of the observed behavior.

There is reason to expect that with better knowledge of the skeletal dynamics the clinician will be able to prevent danger of tetany following removal of the adenoma as he now prevents danger of thyroid storm following surgery in Grave's disease.

4. *Skeletal Electrolyte Depot:* It is proven that large stores of many of the body electrolytes exist in bone and enter or leave the skeleton in significant quantities in health and during disease. This knowledge is largely academic because we do not understand the mechanisms by which transfer occurs and so cannot manipulate them to suit the patient's needs. As with most other important things of which we are ignorant, it is difficult to foresee the need for consideration of such a topic at clinical or basic science levels. Yet better knowledge of the skeleton's contribution to the pathophysiology of neuromuscular irritability, serum sodium and chloride levels, serum calcium and phosphate levels, parathormone increase, citrate metabolism and heterotopic calcification should lead to better diagnosis, treatment and understanding of numerous related diseases.

The hydroxyapatite-hydration shell theory alone cannot explain why the physiological ions behave so differently in different ages, different sexes, different diseases and even among the different ions. Worker A finds that X ion is firmly fixed in vitro in the crystal lattice while worker B reports that in vivo it is loosely bound and readily exchangeable!

CONCLUSION

The first step in injecting some order into the present jumbled state of affairs is to *recognize* the major factors which affect blood-bone interchange as it applies to ionic or molecular substances. (By blood-bone interchange is meant transfer to or from the skeleton from or to the blood). Recognition of such factors will lead to *qualitative* understanding of events. Following this, *quantitative* understanding will come. The factors to be discussed, although not complete, are a beginning in recognition.

The factors to be discussed fall naturally into 3 classes: physico-chemical, structural and biological. Their separate consideration is somewhat artificial since their effects and occasionally their genesis are interrelated. The strategem of separate consideration is needed for clarity at present.

The fundamental unit of structure and of interchange in bone is the hydroxyapatite-hydration shell complex. The elaboration of this concept is a major advance in our understanding of skeletal behavior and previous remarks should not be interpreted as criticism of this theory. The manner in which it is usually applied in explaining interchange features *is* criticized. A considerable group of characteristics affect the ability of an ion to get from the extravascular fluid to a hydroxyapatite-hydration shell complex to such an extent that effects occur which are quantitatively more important than the mass of crystallites themselves.

The current hydroxyapatite-hydration shell theory will be summarized first. The factors affecting the ability of the total complex to equilibrate with the extravascular fluid will then be considered separately. A familiarity with bone histology will be assumed.

HYDROXYAPATITE HYDRATION SHELL COMPLEX[13]

Of the two major skeletal phases - mineral and matrix - the mineral is quantitatively most important in blood-bone interchange. The matrix will henceforth be ignored although it does have some properties related to the present theme and although in some manner it is essential to the deposition of mineral in bone.

The hydroxyapatite crystal is an arrangement of atoms in space: a lattice. It is not a chemical compound, various substitutions in chemical identity being possible while the same lattice is preserved. The crystallite is about 300 Å long, needle shaped and has the approximate chemical composition of $Ca_{10}(PO_4)_6OH_2$ in our skeletons. Thus a molar Ca/PO_4 ratio of about 1.7 should exist. In fact ratios of 1.6-2.2 are found. This discrepancy will be explained later.

The hydroxyapatite crystal is somehow bound to the surface of collagen fibers and the binding is related to the 640 Å periodicity of the collagen. While some crystals may appear isolated in the matrix, 95% of the matrix is collagen so most of the crystals are bound to or in collagen bundles. In unmineralized matrix a large amount of water is present but in highly mineralized matrix little water is present, water being displaced by the crystallites. The amount of matrix probably is constant during this relative shift.[14]

The surface of the crystallites strongly bind several layers of water and some adventitious ions. More loosely bound to this inner layer is a thicker outer layer consisting of more water and adventitious ions. The crystal is thus surrounded by a hydration shell. The enclosed crystal gives us the total unit: hydroxyapatite-hydration shell complex.

The chemical composition and relative proportions of one anion or cation to others of like sign may be altered in 5 ways, all of which probably occur in living bone.

a) Ions may be loosely bound in the two layers of the hydration shell.

b) Ions may be adsorbed and strongly bound to the crystal surface.

c) Ions may displace and substitute for atoms in the crystal lattice.

d) Ions may displace an atom in the crystal lattice and leave the position vacant.

e) Ions may intrude into the lattice between the lattice positions and remain there.

In vitro it is known that hydration shell exchange with the solute (a) is rapid, adsorption layer exchange with solute less rapid (b) and lattice effects (c, d, e,) slow. The Mg ions are felt to substitute for calcium ions. Carbonate is felt to be adsorbed or in the hydration shell. The above statements summarize the knowledge leading to various statements in publications suggesting that a particular ion must be in the hydration shell or lattice to explain rate and quantity of exchange with a particular radioisotope in vivo.

Current theory further recognizes that gradients in activity coefficients between the crystal complex and the solute will lead to equilibration by transfer of appropriate amounts of ions. The large crystal surface area per gram of bone (over 200 M^2) provides a large area for adsorption. With the attendant hydration shells the depot capacity of the skeletal mineral is impressive in-vitro. If this were available in-vivo patients would not die of acidosis however.

Recent work has uncovered the disconcerting fact that the problem of the solubility of the hydroxyapatite crystal mass in the body fluids is exceedingly complex. Solubility is affected by temperature, pH, concentration, charge, mass, nature of adventitious ions present, surface active agents, specific ion effects, dielectric constant at crystal-solute interface and others. Empirically it is found that the serum is super-saturated with respect to the bone mineral although the concentration of ionized calcium and phosphate in the serum is below that needed to cause a precipitate. Efforts are currently being made to determine the effect on activity coefficients of a number of physiologic substances, hoping that the effects may provide comprehension.

If the chemical composition of the hydroxyapatite is qualitatively correct then buffering is readily explainable by the release of hydrogen ions and uptake of hydroxyl ions as the solute ions precipitate to form hydroxyapatite. The converse should also be true with removal of hydrogen ions from and addition of hydroxyl ions to the solute as the bone salt dissolves, thus buffering an acidosis. Since it is not known if carbonate is present in the divalent or monovalent form its contribution to buffering is speculative.

Complicating this picture is the fact that evidence exists for the presence of a solid phase of some other lattice structure than hydroxyapatite in living bone but not in dead bone.[2,8]

FACTORS AFFECTING ION TRANSPORT BETWEEN BLOOD AND HYDROXYAPATITE CRYSTAL

PHYSICOCHEMICAL

It should be noted in presenting these factors that they are known to affect *diffusion impedence* in-vitro and in other fields such as chemistry, geology and metallurgy. When direct evidence of the presence of the effect in-vivo in bone is lacking this will be noted.

1. *Ionic Radius:* When bone is mineralized less than about 80% of the possible maximum, it is apparently completely permeable to all ions and molecules of physiologic importance except the (uninvestigated) soluble proteins. This feature is the basis of the writer's fuchsin standard for classification of mineralization densities in fresh, undecalcified sections.[3,4,6,8] As mineralization increases, ions and molecules of decreasing size exhibit, first, diffusion impedence out of proportion to that exhibited by the next smaller ionic radius; second, total exclusion from the bone with further rise in mineral density.[7] In any given moiety of bone mineralized over 80% there is a limiting ionic radius, above which detectable permeation of bone does not occur, below which it does. At 80% mineralization the ionic radius is on the order of fuchsin, methylene blue and similar histological dyes. At 90% urea, uric acid, glucose and citrate are excluded. At between 90% and 95% the heavy metals are excluded.

Tabulations of radioisotope exchange data strongly suggest the operation of the ionic radius factor in in-vivo experiments.[13]

2. *Ionic Charge:* Given the same or nearly the same ionic mass and size, and uniform mineralization density, the valence of the ion exerts an effect. Increasing valence is accompanied by increasing diffusion impedence. The effect seems to be less significant than the ionic radius effect. While the effect is readily demonstrable in vitro in fresh bone, published exchange data do not corroborate due to the lack of similarity in mass and ionic radius of the ions experimented with.

3. *Ionic Mass:* In other fields increasing ionic mass is accompanied by increasing diffusion impedence. It is tempting to assume that the large, rapid in-vivo exchange of skeletal water with the deuteron is the result of its small mass and in part it may be. Its small ionic radius probably accounts for most of the observed permeability however. A mass effect, while to be expected, exists by inference only at present.

4. *Blockade:* In numerous fields it is known that certain ions will produce intentional or objectionable blockade of diffusion, in effect seeming to plug up the interstices which are the entrance to diffusion pathways in a substance. In fresh bone in vitro this effect can be observed with some of the heavy metals and with fluoride. More significantly it can be produced with phosphate ions in pH ranges higher than 7.1.[8] This appears to be a completely uninvestigated bone effect, there being no publications dealing with it. One wonders how much of the skeletal effect of fluorine may be attributable to blockade. In any event blockade, if it occurs in vivo, would be expected to display quantitative effects out of proportion to the variation in concentrations of the active ion or molecule.

STRUCTURAL

These effects are less speculative than the physicochemical effects just listed since they are readily demonstrable and in some cases provide the most suitable explanation for available data.

1. *Mineralization density per unit volume of bone:* From the time newly formed matrix begins to mineralize until it is destroyed by remodelling processes (sometimes after 55 years of existence) progressive accretion of mineral with displacement of water occurs. Initially mineral accretion is rapid so that 80% of maximum mineralization has occurred in about 4 weeks. The accretion rate diminishes so that at one year density is about 90% and at 10 years about 98% of maximum. The mineralization density of bone therefore is a function of the age of the moiety being considered.

Because children are growing and enlarging rapidly, the average age of their skeletons is low, being about two months at birth, 9-12 months at age two and 2-4 years at age 10. (Older bone is removed by remodelling). Their average skeletal mineralization density is similarly low at birth and gradually increases with increasing age.

Adults, having ceased growing, are turning bone over as a result of routine remodelling only and the older they get the greater is the average age and thus mineralization density of the innumerable bone moieties which make up their skeletons.

Correlated with the above are clinical exchange data for various bone-seeking radioisotopes. The younger the individual, the more complete is the exchange of the radioactive tracer with the bone depot of ions. This cannot be explained by hydroxyapatite-hydration shell complexes. It is due to the fact that diffusion impedence is increased with increasing mineralization density. The average rate of skeletal exchange of a given ion in the extracellular fluid is diminished by diffusion impedence. The farther a given crystallite is located from a source of extracellular fluid, the slower is its equilibration with alterations in the extracellular fluid. Since most exchange experiments cover a few hours or at the most a few days, in adults (animals and man) a good share of the skeleton completely escapes equilibration with the blood.

2. *Diffusion Polarization:* Histologists have long felt that the lamellar structure of mature bone does not reflect any significant difference in structure or function. For the purposes of ion diffusion impedence this feeling is false. In undecalcified sections lamellae are biphasic. Zone A is thin (about 2 u), highly refractile, homogeneous and has the longer optical path. It corresponds to the bright band under polarized light and the zone of collagen bundle interweaving in silver stained, decalcified sections.

Zone B is thicker (about 5 u), under phase contrast appears composed of irregularly placed blocks of material jumbled together and is the dark band of polarized light and the zone of unidirectional collagen bundles in silver stained, decalcified sections.

In-vitro in perfectly fresh, unfixed, undecalcified, wet sections prepared by the writer's techniques, Zone A has an appreciably higher diffusion impedence than Zone B, diffusion progressing twice as far in the latter. This behavior is manifested with

heavy metals, with dilute acid buffers and acids, with alkaline doubly ionized sulfide, with progressive permanganate oxidation and with various leaching and complexing techniques.[7,8]

Accordingly the lamellar structure of the normal skeleton probably creates diffusion polarization so that ions diffuse more readily in Zone B and parallel to it than in or across Zone A. This effect is not present when mineralization is below 80-85% of maximum and so does not exist in new forming bone or in the condition called feathering.[6]

A similar but more effective diffusion barrier occurs in the cement line. In fresh undecalcified sections the cement line is also biphasic. The portion lying in the older bone mass is highly refractile, optically of longer path length, more densely mineralized and contains more alizarin-stainable calcium than the portion lying in the newer bone. The latter is part of the new bone and was cemented down on the older part when the formation of the newer bone began. It is collagen poor and poor in alizarin-stainable calcium. While it is as permeable as lamellar zone A, the denser part lying in the older bone is extremely impermeable and probably polarizes ion diffusion also.[8] Under pathological circumstances the diffusion impedence of cement lines creates significant effects which will be outlined later. (See Figs. 1, 2, and 3).

Figure 1

Undecalcified cross section human clavicle. Etched with 1 M Na Cl, stained with basic fuchsin. The etching removed bone salt from the dark areas permitting subsequent stain of the exposed matrix. The lamellar orientation is apparent. The escape of the cement lines is especially striking.

Figure 2

Longitudinal section human rib. 0.1 N $AgNO_3$ one month. The lamellar orientation of the diffusing silver ions is evident. There would appear to be an even finer diffusion structure than the lamellar one discussed.

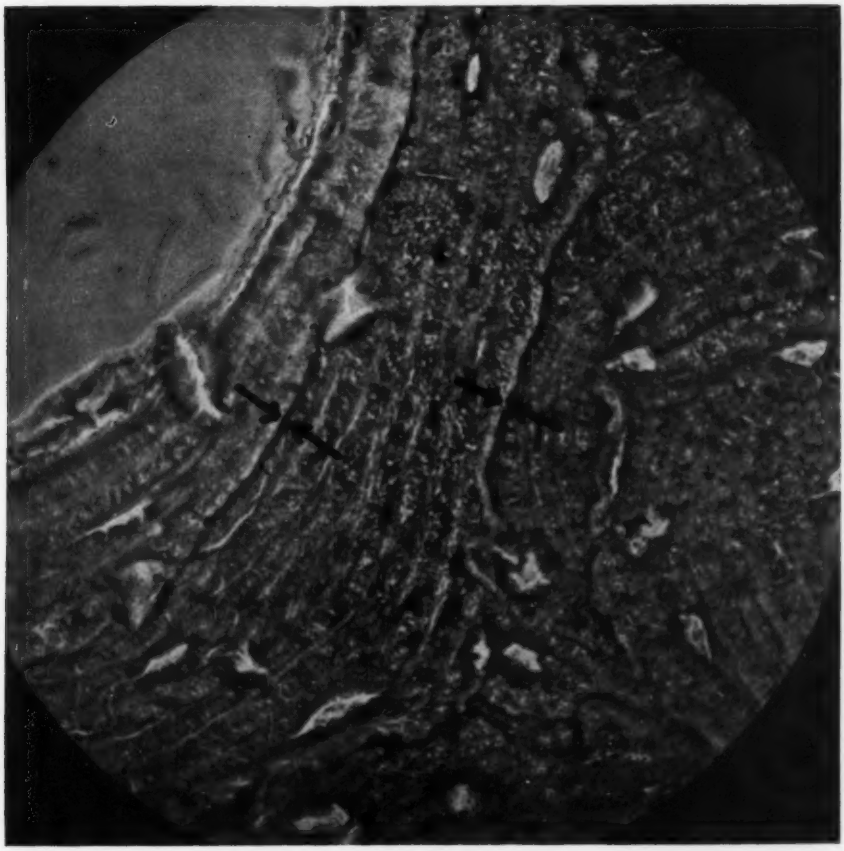

Figure 3

Phase contrast photomicrograph of fresh human tibia, cross section. About 500 X. The lamellar structure discussed in the text is revealed. The coarser lines between arrows are cement lines and the biphasic structure can be seen.

3. *Canalicular network:* Fortunately for healthy people normal bone contains an extensive canalicular network which connects lacunae to vascular channels and thus a source of blood. There are on the average 26,000 lacunae and 1,000,000 canaliculae per mm^3 in human bone and on the average no crystallite is further than about 5 u from a canalicula or lacuna.[8] This is a very elaborate distributional network. In addition there are normally numerous anastomoses across cement lines so that the diffusion barrier is normally circumvented.

All is still not rosy. In the absence of life diffusion along the canalicular network is poor, whether it be determined in vitro with various reagents or in vivo with substances such as tetracyclines. Days are required for reagents to diffuse from the Haversian canal to the outermost lacunae, a distance averaging 100 u. The reasons

for this slow diffusion are physicochemical in nature and due basically to the smallness of the canalicular lumen, 0.35 u in diameter. At this size the charge, surface tension and dielectric constant at the canalicular wall exert significant effects. With this note the biological effects are introduced. (See Fig. 4).

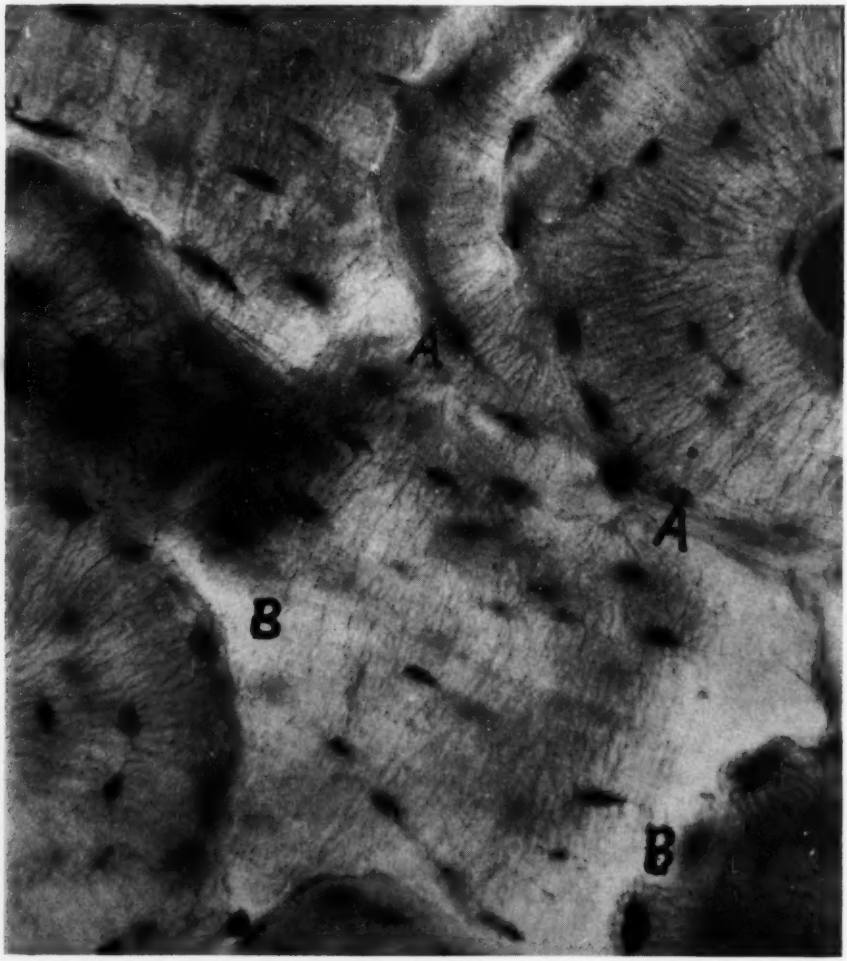

Figure 4

Cross section human rib about 800 X, basic fuchsin. The innumerable canaliculae are impressive in spite of the narrow depth of focus of the objective used in taking this photomicrograph.

BIOLOGICAL EFFECTS

These effects divide naturally into normal and pathological.

Normal

1. *The Metabolic Pump:* Normally the lacunae contain living osteocytes. The

metabolism of these cells creates concentration gradients between the fluid around the bone's blood vessels and the fluid bathing the osteocytes in the lacunae. The maintenance of these gradients provides the necessary energy and osmotic pressure to accelerate ion diffusion betwen blood and osteocyte. Because of the impermeability of the surrounding bone the diffusion pathway is the canalicular lumen.

Because gradients are produced it is necessary to infer that the composition of the fluid in the lacuna is different from the composition of the fluid around the bone's blood vessels. The differences could involve molarity, pH, activity coefficients and chemical composition. A temperature difference might be anticipated.[15]

The possible differences in the lacunar fluid as compared to the extracellular fluid and serum represent a large field of ignorance and future research which must be delved into before osteocyte metabolism and bone salt composition can be properly understood. The osteocyte in effect is the keeper of the gate between bone and blood. If we wish to control the gate we must learn the coinage of the keeper.

2. *Halo Volume:* In normal, living bone the bone wall of the osteocyte lacuna and of the parts of the canaliculae within about 2 u of the lacuna is normally more permeable than the remaining intercanalicular and interlacunar bone. In some fashion this increased permeability is produced and maintained by the metabolic effect of the osteocyte on its bathing lacunar fluid. When the cell dies the halo volume permeability disappears, in vitro as well as in vivo.[8,9,16]

Halo volume is about 15% of the skeletal volume exclusive of marrow and vascular channel. It represents a readily permeable fraction of the skeleton situated close to the metabolic pump and canalicular network which enables it to equilibrate rapidly with short-time changes in the blood. Most of the rapid exchange of bone with blood probably occurs in halo volume. Its ready permeability is the reason that initial exchange rates are so high. If the halo volume were absent the initial exchange rates should be much slower, on the order of the rates and quantities found in dead bone in vitro. This point has been studiously ignored in presentation of exchange data interpretation. (See Fig. 5).

3. *New Bone Formation:* New-forming bone is permeable because it has not had sufficient time to mineralize up to the 80% mark at which ionic radius, charge and mass of diffusing ion seem to become important. The amount of new bone in the skeleton represents a readily available, rapidly and nearly completely exchangeable depot of bone mineral. From the standpoint of quantity new bone comprises about 40% of the new born skeleton, 10% at 18 months and 0.05% at age 40. It comprises less of the total skeletal mass however due to the fact that old bone has more mineral per unit volume than new. As a factor in blood-bone interchange new bone would be important in the very young and decrease in importance with age, probably being unimportant in comparison with other effects after age 10.

Pathological

1. *Feathering:* In this condition abnormal amounts of incompletely mineralized, fuchsin permeable bone accumulate in the skeleton, especially in symptomatic osteoporotics. Its incidence (over 15% of the skeleton affected) in a general hospital

Figure 5

Cross section human fibula. The patient had Vitamin D resistant rickets. The large zone of fuchsin permeability around many of the osteocytes is abnormal and may be compared with Figure 4. This is the Halo Volume part of the bone. Basic fuchsin, about 400X.

patient population is about 10%. Because of canalicular occlusion at the wall of the Haversian canal not all feathered bone is readily available to the extravascular fluids but significant quantities of it are. The presence of feathering may be expected to affect blood-bone interchange rates and quantities in the occasional patient who is affected by it. (See Fig. 6).[4]

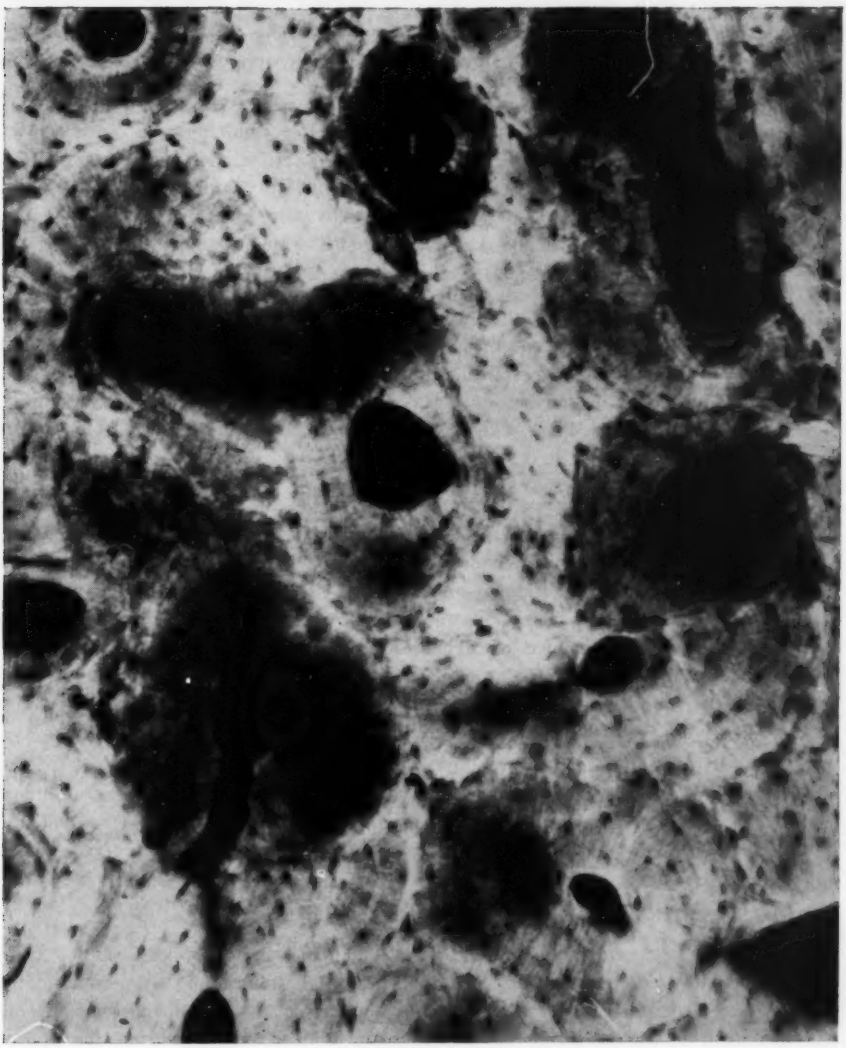

Figure 6

Feathered bone from the tibia of a 48 year old woman. The stained parts of the Haversian systems illustrated are fuchsin permeable and incompletely mineralized. About 200X, basic fuchsin.

2. *Osteocyte Death in Vivo:* Normally increasing numbers of osteocytes die as we age, on the average 50% of them being dead at age 70. There are wide variations around this average, some of this variation being pathologic. Some skeletons appear to be over 80% dead. The absence of the metabolic pump in the affected patients who are for the most part elderly would be expected to affect blood-bone interchange

processes. Since halo volume are lacking around these dead cells the variability of certain exchange data in older humans would appear to be correlated with osteocyte death and halo volume loss rather than with mysterious alterations in the hydroxyapatite crystals.[5,10]

3. *Micropetrosis:* In this condition a peculiar hypermineralization is found in the bones, mainly in older patients. Canaliculae are occluded with mineral and often lacunae are similarly occluded. The affected osteocytes are dead. Micropetrotic bone is dead, hard, brittle, very highly mineralized, extremely impermeable in-vitro and exists on an average of 15% of the skeleton at age 70, wide variations again occurring. Some skeletons on hand are over 40% micropetrotic.

Micropetrotic bone does not partake in ion exchange with the blood to any significant degree in time periods of under two months. The occasional patient who exhibits this change in his bones would thus exhibit abnormal blood-bone exchange phenomena. The affected portion of his skeleton would be isolated by the impermeability of the affected bone and of the cement lines which sharply separate it from normal bone.

While micropetrosis of severe degree is not common its first stage, occlusion of the canalicular anastomoses across cement lines by mineral, is quite common after age 50 and increases with advancing age. The effect of this minute histological change is that progressive isolation of extraHaversian bone from the Haversian vessels occurs, markedly impeding rate and quantity of equilibration in activity gradients between these two units. Since extraHaversian bone comprises about 60% of the average elderly skeleton this effect is not a minor one. (See Fig. 7).[4]

DISCUSSION

The factors just outlined are a start towards recognition and measurement of the aids and hindrances nature has placed between extravascular fluid and bone crystallites. Their large potential effects and in several instances the corroborative in-vivo evidence of their existence make it unwise to ignore them when interpreting observed blood-bone exchange data.

The material presented may be summarized as follows:

The basic depot and exchange unit of the skeleton is the hydroxyapatite-hydration shell complex with its adventitious ions. Between this complex and the extravascular fluid in bone's vascular spaces three groups of effects occur which either impede or aid movement of an ion from the extravascular fluid to the vicinity of the crystal complex. The physicochemical factors include the size, charge and mass of the diffusing ion and a blockade effect. The structural factors include mineralization density, diffusion polarization and the canalicular network. The biological factors include, normally, the osteocyte's metabolic pump, halo volume and new bone formation while pathologically feathering, osteocyte death and micropetrosis are found.

It has already been mentioned that separation of these effects is artificial. No attempt has been made to outline the mechanisms of the effects or to claim that the list is complete.

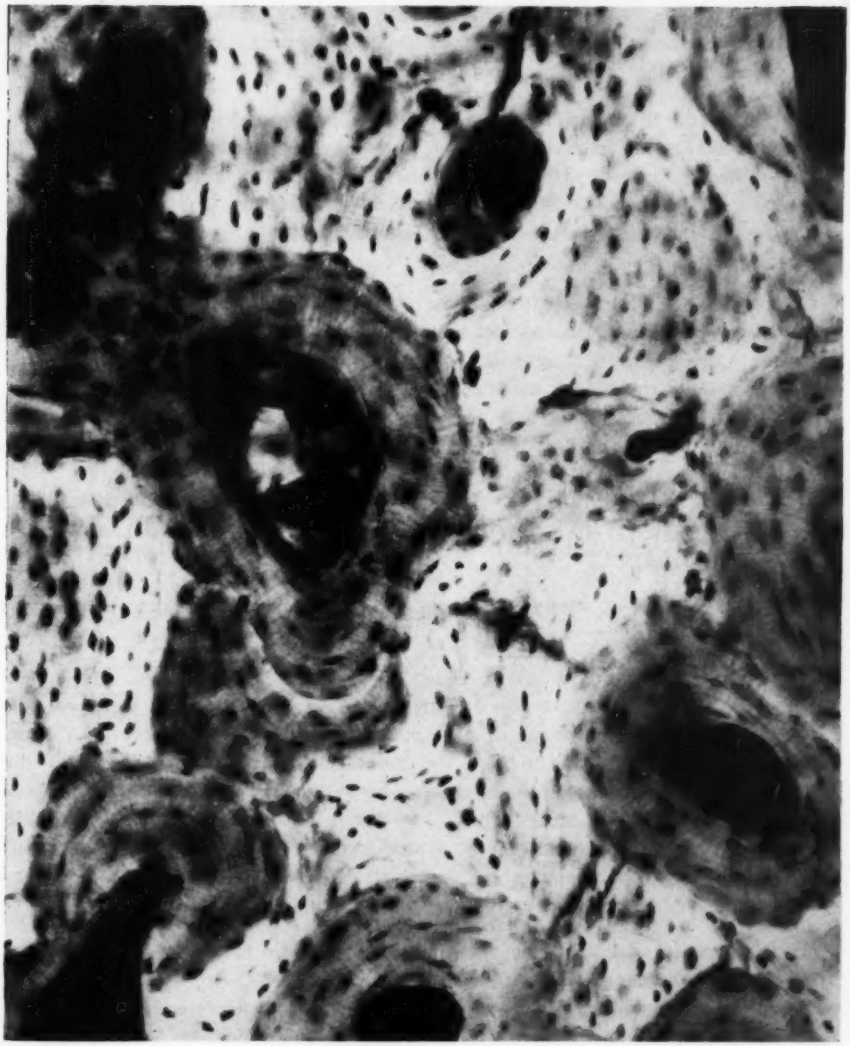

Figure 7

Micropetrosis. Human femur, 47 year old woman. Cross section of the cortex. The white areas appear so because their canaliculae are filled with mineral and unstainable. The Haversian systems and small parts of the interstitial lamellae appear darker because of stained lacunae and canaliculae out of focus above and below the focal plane of the objective.

We do not yet know enough about bone to propose a comprehensive theory of its interactions with the extravascular fluids. It has been common in similar situations that the afflicted field is beset with schools of thought and with narrow ranges of interest which are zealously guarded. While there is general agreement that something

new is needed, there is general consternation when it arrives! The field of the interaction of bone with the extravascular fluids is now in such a state. It is with this in mind that the following remarks are recorded.

If one feels that calcium turnover in osteoporotics should be low, he has available a number of papers which will substantiate his belief. If he belongs to another school and feels that it is high, he may find papers to substantiate this. How can such diametrically opposed conclusions be reconciled by one who belongs to the school of objective evidence, if hydroxyapatite is nature's sole exchange mechanism?

The same situation exists elsewhere. Phosphate bone exchange from 0.5% to 40% can be found, as can calcium: 0.5% - 30%; sodium: 0.1% - 75%; deuterium: 50% - 100%; carbonate: 1% - 60%. Widely varying exchange data can be found for states such as osteomyelitis, rickets, osteomalacia, Paget's disease, cardiac disease, renal disease and no disease.

Publications can be found which conclude (as wished) that bone is normal or abnormal in quality in various diseases, normal or abnormal in amount, hard or soft, permeable or impermeable, new or old, live or dead. It has been frequently written that the osteocyte maintains the bone, occasionlly that it has no function but rarely has any specific function been attributed to it although it is becoming evident that because of where it sits and of what happens or does not happen, it does something.

It is time this state of affairs ended. Exchange data can mean little unless the sex, age, type of bone examined, method of examination and its limitations, physiological state of the organism, physiological or pathological state of the bone, concomitant treatment "unrelated" to the experiment and the permeability of the bone are specified. Calcium balance studies have limited value unless it is known whether the calcium loss occurs from bone destruction (osteoclastic activity), halo volume halisteresis or migration out of the crystal complexes. The same holds true for positive calcium balance and for balance studies on magnesium, phosphate, sodium, carbonate, lead and fluorine.

The processes of osteoclastic resorption, halo volume halisteresis and crystallite exchange are different with different metabolic controls. It is folly to avoid measuring them and instead interpret their gross effects in a currently popular vein. Bone mineral is, in any final analysis, the material affected by skeletal gain or loss; it is injudicious to avoid studying it by decalcifying the bone and promptly forgetting the mineral. When this is done half of the information in the original bone is flushed down the drain. When a metallurgist or geologist knows that diffusion through different phase systems is governed by specific effects, it is unwise to exclude consideration of these effects in bone simply because they have not been completely investigated or quantitated. When a microscopist is able to demonstrate physical states in bone which impede or accelerate ion diffusion it is foolish to ignore the states because their effects might invalidate the interpretation of data in the currently popular vein. When exchange rates consistently reveal an initial

large exchange rate and a subsequent small one in the face of a physical situation which, according to current belief, should lead to steady rate exchange, it is folly to ignore it.

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COMPARATIVE STUDIES OF HUMAN MELANOCYTES IN SKINS OF VARYING DEGREE OF PIGMENTATION*

FUNAN HU, M.D.** and PATRICIA F. LESNEY**

The color of human skin appears to be dependent on a number of factors which include (a) the thickness of epidermis, (b) amount of blood supply and degree of vasodilation, and (c) amount of pigments. On the basis of their studies, Edwards and Duntley[1] state that the color of normal human skin takes its origin from five primary pigments, i.e., melanin, oxyhemoglobin, reduced hemoglobin, carotene and melanoid. The amount of melanin pigment in the skin, however, is the chief factor which determines the degree of coloration of the human skin of different races.

In an earlier paper,[2] the difference in morphology and staining reactions of the melanocytes of darkly and lightly pigmented skin was discussed. This comparison was extended further in this study.

MATERIALS AND METHODS

The preputial skin of infants of varying degree of pigmentation was explanted in vitro with the roller tube tissue culture method.[3] At the end of three to four weeks incubation, when usually epithelial sheet outgrowth was established, the explants which were embedded in the plasma clot on the coverglass were removed from the culture tube to be processed for cytological examinations. The topmost part of the explant which consisted of the keratin and superficial layers of the epidermis of the skin became loosened from the underlying epithelium and could be removed by careful lifting of the part with a pair of fine forceps. The remaining explants together with the outgrowing epithelial sheets were then ready for various histochemical studies which included dopa,[4] silver,[5] gold,[6] May-Gruenwald-Giemsa[7] and supravital methylene blue staining.[8]

RESULTS AND DISCUSSIONS

Among the various staining procedures employed in this study, the ammoniacal silver nitrate technic described by Masson but modified slightly in our laboratory for tissue culture preparations proved to be the best. The melanin pigment in melanocytes, epithelial cells and occasional fibrocytes in the culture was visualized in the form of black silver deposits. The melanocytes in the original skin explants were found to be invariably larger and more polydendritic although they may or may not be more darkly stained than those in the outgrowing epithelial sheets (Fig. 1-3).

Although the differences in morphology and staining reaction of the melanocytes were not very apparent in skins which varied only slightly in their degree of pigmentation, the melanocytes of the darkly pigmented skin were always larger, more dendritic with secondary branching and more strongly argentaphilic than those of the lighter colored skin. The amount of melanin pigment was also found to be more in the epithelial cells of the darkly pigmented skins (Fig. 3, 4). These findings confirm the observations of Hu et al[2] in that the functional activity of the pigment cells correlated with the size of the cells, their shape, and complexity of their dendrites.

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Figure 1

Negro skin culture. Silver. 100X. Original skin explant at the right lower corner. Remainder is outgrown epithelial sheet with many melanocytes in between. The epithelial cells are only barely visible. their intracytoplasmic melanin granules which stained black help to outline the position of their nuclei.

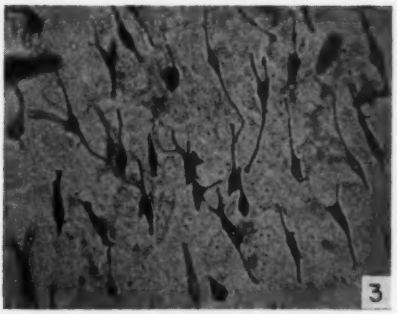

Figure 3

Negro skin culture. Melanocytes in the outgrowing epithelial sheet. Silver. Phase microscopy. 430X. The melanocytes in this area are smaller and morphologically less complex with bipolar or tripolar dendrites in contrast to those in the explant portion where they are mostly polydendritic. Also note the unstained epithelial cells in the background with perinuclear silver-positive melanin granules.

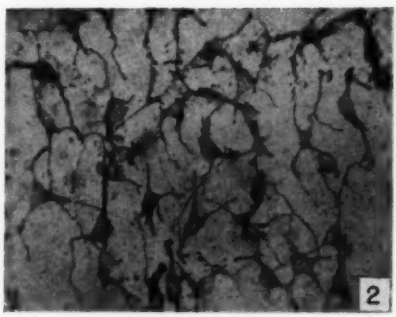

Figure 2

Negro skin culture. Silver. 430X. This shows the "syncytial" arrangement of the large polydendritic melanocytes in the skin explant after removal of the topmost layers of epidermis.

Figure 4

White skin culture. Melanocytes in the outgrowing epithelial sheet. Silver. Phase microscopy. 430X. Compare with Fig. 3. The melanocytes are definitely smaller and less dendritic. Note also the scarcity of silver-positive granules both in the melanocytes and in the epithelial cells.

Staricco[9] demonstrated larger and more dendritic melanocytes in skin which was treated locally with thorium X. This again confirms the correlation between the pigment-forming activity and the morphology of the pigment cells.

CONCLUSIONS

Our study indicated a definite correlation between the size and morphology of the melanocytes and their pigment forming activities. This basic information may

be utilized to evaluate the effects of agents which either stimulate or inhibit this specific function of the pigment cells.

The authors express their appreciation to the staffs in the Department of Obstetrics and Delivery Rooms for their cooperation in supplying the skin specimens used for this study.

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THE ROLE OF IONS IN MUSCLE EXCITATON AND CONTRACTON

RAYMOND E. FULLER, M.D.* and WILLIAM L. MORGAN, JR., M.D.*

The cell membrane is vital in establishing and maintaining an intracellular ion concentration that is different from that of extracellular fluid. Because of membrane selective permeability to ion flow, such gradients exist and are the basis for the electrical potentials generated within the membrane. In specialized tissues, such as nerve and muscle, the additional properties of excitability and conductivity are highly developed. Newer techniques, especially work with radioactive isotopes, have helped in understanding the action of ions in cellular physiology. It is the purpose of this paper to review the more recent theories of the role of ions in muscle excitation and conduction.

THE CELL MEMBRANE

The sarcolemma (cell membrane of muscle fiber) discussed here is not the inert histologic structure which encloses the cell. It is a living dynamic barrier that possesses properties of selective permeability.

The membrane itself is composed of a few layers of protein molecules overlying a thin layer of lipoid material. The exact structure of the membrane has not been defined. Some believe it is sieve-like with pores of varying sizes, accounting for its selective permeability. Eyring and Parlin, on the other hand, describe the membrane as being composed of lipoid material between layers of simple protein molecules, which are coiled into spirals. These protein spirals sit on either side of the thin lipoid layer like upright barrels. In the resting state, the spirals orient themselves so that the positively charged amino end groups are directed toward the negative charge of the cytoplasm, whereas the negatively charged carboxyl end groups are directed toward the extracellular fluid.

The electron microscope reveals an outer reticulum of fine fibrils and an inner structureless membrane, the combined system being 100-200 A° in thickness. Ridges are also seen on the inner surface of the sarcolemma, to which the Z lines attach. Recently Huxley and Taylor have suggested that the transmission of membrane depolarization may be conveyed to the cell interior by means of these lines.

Although the structure of the sarcolemma has not been correlated with its function, the membrane demonstrates three main properties. There is (1) selective permeability to ions and other substances, and it is (2) the site of an electric potential as well as (3) a conductor of the wave of excitation.

It is believed that the sarcolemma obtains energy for its various functions from oxidative metabolism occurring within the mitochrondria of the cell.

The membrane selects ions according to their size and charge. It therefore controls the quantity of ion movement (or flux), as well as their direction of flow. The membrane potential which governs this selectivity is in turn dependent on ionic gradients to maintain a resting potential. With a drop in the resting potential selectivity

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is decreased, and the membrane becomes more permeable. This change in potential (depolarization) initiates the contraction of the muscle cell as well as the propagation of the electrical charge along the length of the fiber.

SODIUM AND POTASSIUM FLUX

Deane and Smith found that in man the intracellular potassium ion concentration is 112 mEq./liter of intracellular water and the sodium ion concentration is 37.0 mEq./liter. Comparable concentrations occur in animals. Conway showed that half of the intracellular sodium in frog muscle is interstitial. The other half is located within the fibers as internal fiber sodium and external fiber sodium. The site of the external fiber sodium is thought to be the sarcolemma, where the proteins have a pH similar to plasma and therefore exhibit a preponderance of negative charges. Because of this, the sarcolemma could remove potassium and sodium ions by acting as a resin. Current theory argues that fluxes of sodium and potassium occurring with depolarization and repolarization of the membrane take place only in a limited area about the membrane.

Small ions pass the cell membrane with varying degrees of freedom. The critical size for rapid passage of cations is that of the potassium ion. Thus potassium and rubidium ions enter the cell at appreciably fast rates. Sodium and lithium are slower, because these hydrated ions are larger than the hydrated potassium ion. Potassium travels from 24-100 times more rapidly than sodium, depending on the state of the membrane. Anions such as chloride, bromide, and nitrate enter freely, but bicarbonate ions diffuse very slowly and sulfate ions are practically excluded. Calcium and magnesium may enter the cell by forming un-ionized complexes with organic compounds, but this may not be true when the membrane is depolarized.

THE SODIUM PUMP

For many years physiologists have been fascinated by the distribution of intracellular and extracellular sodium and potassium. Many theories have been postulated to account for the mechanisms involved in these ionic distributions.

The sieve theory of Boyle and Conway assumed that the selective permeability of the membrane was due to pore size alone and not to electrical charges. Therefore the membrane would be more permeable to the smaller potassium ion than the larger sodium ion.

Later, radioactive istope studies showed that the cell is permeable to a great many so-called "impermeable" ions such as sodium, calcium, magnesium, phosphorous and lactate. The studies of Hodgkin and Horowicz, using radioactive Na^{24} and K^{42}, demonstrated an exchange of intracellular and extracellular cations. The sodium ion exchange was less than the potassium ion exchange in the resting state.

Ussing has tried to explain the above by his "exchange diffusion" mechanism in which a carrier system in the membrane transports sodium into and out of the cell. It is a coupled reaction so that as one ion is carried in, another is carried out. This does not, however, explain the low intracellular sodium. Ling in his "fixed charge hypothesis", has dispensed with the concept of an external membrane and maintains that the relative degree of ionic accumulation within cells depends on

the comparative energies of adsorption of the various ions onto fixed charges of the intracellular proteins. Ions such as potassium are strongly adsorbed and accumulate to a greater extent than ions such as sodium, with a lower energy of adsorption. Russian authors believe that sodium is less soluble in the cytoplasm than potassium and that this accounts for the lower concentration of sodium in the cell.

The majority of investigators today believe that the basis of the low sodium concentration within the cell is due to two mechanisms: 1) the relative impermeability of the cell membrane to sodium, and 2) an active extrusion of sodium by a pumping mechanism. A sodium pumping mechanism is deemed necessary since sodium must be extruded from the fibers against both a concentration gradient and an electrical gradient.

Conway believes that the sodium pump utilizes a transport mechanism by means of a cytochrome system similar to the hydrogen ion transport in oxidative reactions. Evidence supporting this is that the net extrusion of sodium was completely inhibited by anoxia or cyanide in the immersion fluid. Critics of the sodium pump theory claim it would take a prohibitive amount of the cellular metabolism to supply the pump with energy. Lewis and Ussing had estimated this to be as high as 30%. Keynes, however, found that the sodium pump may only require 10% of energy available from resting metabolism. He also concluded that sodium efflux must be coupled with potassium influx in a cycle carrying a potassium ion in for each sodium ion leaving, thereby reducing total energy expenditure. Other evidence to support this is that low sodium efflux occurs in potassium-free solutions, whereas potassium-rich solutions cause increased sodium efflux with a proportionate increase in oxygen consumption.

Another concept of the sodium pump is that of Parlin and Eyring. According to them, sodium influx at the time of depolarization initiates a period of marked chemical activity forming a metabolite that acts as a carrier to transport excess sodium out of the cell. Sodium pumping therefore results from a union of sodium with this metabolite (P) to form NaP inside the cell. The NaP complex readily diffuses through the membrane and, being more concentrated inside the cell than out, causes a strong outward positive sodium current. The exact nature of the metabolite has not been defined, but Eyring and Dougherty suggest one candidate for the role of pump substance may be histamine.

THE RESTING MEMBRANE POTENTIAL

Classically the resting membrane potential has been explained by the marked difference in concentration between intracellular potassium (Ki) and extracellular potassium (K_e), in a Donnan equilibrium. This distribution of potassium probably occurs in all cells of the body and it reflects the selective permeability of the cell membrane for potassium.

Hodgkin and Huxley postulated that the resting potential is the result of a dynamic type of Donnan system in which the potassium ratio (Ki K_e) is secondary to the existance of a sodium pump. Grundfest also accepted the importance of the sodium pump and further postulated that the resting potential is generated by the

membrane itself. He believed that the resting potential due to the Donnan system *alone* is not tenable and that it must be due to other mechanisms. Some of the evidence he presented follows: 1) The resting potential is found experimentally to be consistently lower than that calculated on the basis of the Donnan ratio. 2) In nerve isolated 10-18 hours, one observes considerable potassium loss and sodium gain, but the resting potential remains the same. 3) Large alterations in intracellular potassium and chloride by microinjection into the squid giant axon did not affect the resting potential until the membrane metabolic activity was disturbed resulting in a decreased action potential and blocked wave propagation. 4) Similar experiments that varied the external potassium concentration led to an alteration in resting potential only after the occurrance of changes in concentration sufficient to alter membrane metabolism.

THE ACTION POTENTIAL

The action potential is associated with a drop in the resting membrane potential below a critical level. When the resting potential is caused to fall below this level, either by an external current or by depression of certain metabolic activities (i.e., the sodium pump), the membrane becomes much more permeable to sodium, potassium, and chloride and these ions flow in the direction of their concentration gradients.

Nachmansohn and others have presented evidence that acetylcholine may be responsible for the increased sodium permeability of the nerve cell membrane. In the resting state acetylcholine is bound to protein or lipoprotein. They postulate that with excitation acetylcholine is released from the binding site and attaches to receptor proteins in the membrane. This combination would change the configuration of the protein and increase the permeability to allow sodium ion influx.

A relatively large influx of sodium ions produces a positive inward current and is thought to be responsible for the sharply rising action spike in the monophasic tracing and the QRS complex in the standard EKG (Hodgkin & Huxley, Keynes). As seen in the diagram, the action spike overshoots the base line, the overshoot actually being a reversal of polarity within the cell from negativity during the resting state to positivity during the height of the positive sodium influx.

During the descending limb of the action potential there is an outflow of potassium ions which initiates the return of the membrane to the resting state. This positive outward potassium current reduces the positive charge within the cell, therefore helping to restore resting membrane potential and decreases the membrane permeability to sodium. Cardiac muscle differs from skeletal muscle and nerve in that the action potential is from 10-100 times longer (up to 300 msec.). This is probably due to the delayed potassium outflow during the early phases of the action potential and slower sodium efflux with a resultant slower recovery phase. Wilde, by means of radioactive tracer studies, showed that the potassium efflux in the slowly beating turtle heart reached its peak coincident with the end of the T wave. As the action potential terminates, the decreasing membrane permeability to sodium influx and the extrusion of sodium by the pump mechanism complete the restoration of the resting membrane potential.

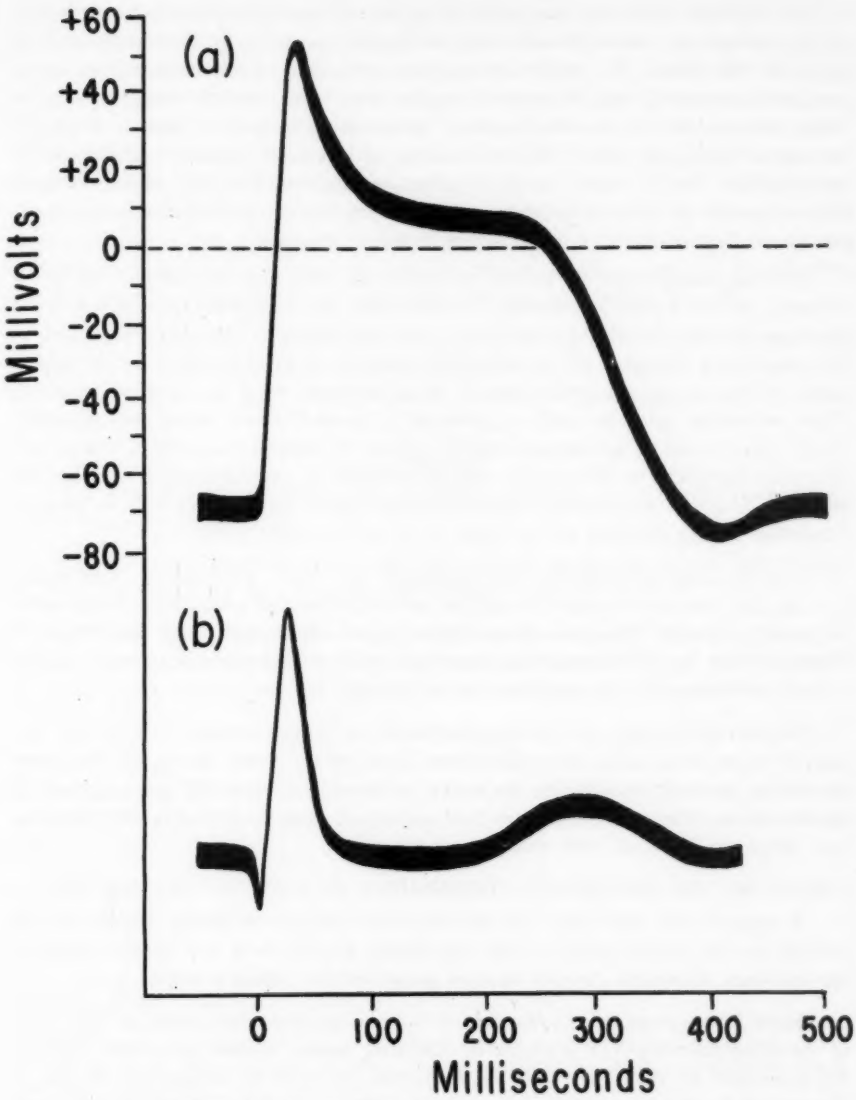

Diagram of a transmembrane action potential (a) as correlated with the standard electrocardiogram (b).

Since the action spike is due to the inward sodium flux, the height of the action potential is dependent on the extracellular sodium concentration. The rising limb becomes lower with decreased concentrations of extracellular sodium. The frog ventricle becomes inexcitable when the extracellular concentration of sodium is reduced to 15% of normal.

As is shown above, the potassium ion plays an important role in the later events of the monophasic action potential and in the generation of the T and possibly U waves of the EKG. At higher extracellular potassium concentrations the action potential is shortened, and the converse applies with below normal concentations. The effects are twofold: 1) increased external potassium concentration leads to a greater concentration of ions within the cell resulting in a greater potassium efflux during repolarization, and 2) higher external potassium concentrations also cause increased efflux of sodium by coupled ion transport so that the rate of repolarization is increased, thus shortening the duration of the action potential (Keynes).

Calcium antagonizes the effects of potassium. This may be due in part to its influence on membrane permeability. Cardiac rate and excitability are known to be depressed by calcium, vagal stimulation, and acetylcholine. This may be related to their depressing influence on the threshold potential of the membrane at pacemaker areas. In these areas throughout diastole or in presystole there is a gradual reduction (loss) of resting potential until a threshold is reached where rapid sodium influx occurs, thus producing spontaneous depolarization. In isolated frog's heart, Hutter and Trautwein were able to increase the resting potential by vagal stimulation so that the threshold level was not reached, thus producing asystole. Others were able to increase membrane resting potential by as much as 33 mv by vagal stimili.

Certain compounds stabilize the membrane and "lock" polarization. This results in a cell that does not conduct an impulse but which remains excitable by maintaining its resting potential. Weidmann demonstrated these effects with local anesthetics on Purkinje fibers. In addition to local anesthetics such as procaine and procaine amide, certain antihistamines and quinidine act as blocking agents.

Digitalis profoundly affects the membrane in cardiac muscle, and indeed this may be its principal effect in cardiac tissue. Stutz, et al., found that it did not affect the resting potential but affected the action potential by diminishing the amplitude of depolarization with loss of overshoot and shortened repolarization. Digitalis therefore may affect the fluxes of both sodium and potassium.

ROLE OF THE MEMBRANE PHENOMENA IN MUSCLE CONTRACTION

It appears well established that the intracellular events in muscle contraction are initiated by the depolarization of the membrane. Exactly how this occurs, however, has not been delineated. Several theories postulated are outlined below.

Some investigators believe the flux of sodium and potassium cause an activation of the actomyosin-Mg-ATP complex by liberating bound magnesium which catalyzes the hydrolysis of ATP. The rate of hydrolysis of ATP by actomyosin in situ is determined by the level of free magnesium in the sarcoplasm. With optimal levels of free magnesium the rate of ATP hydrolysis increases with resultant muscle contraction. Relaxation is produced when hydrolysis of ATP is inhibited by the binding of free magnesium to organic compounds, such as the Marsh-Bendall factor.

Another theory can be postulated for the sodium and potassium flux as related to muscle contraction. Sodium stimulates the dephosphorylation of ATP and ADP in the presence of magnesium. This would result in muscle contraction.

Others have proposed that the entry of calcium during membrane depolarization initiates contraction of the muscle fibers. Microinjection of minute amounts of calcium produces contraction. Bianchi and Shanes have shown by using radioactive Ca^{45} that there is a 30-fold greater influx of calcium during activity than at rest. Calcium is apparently released from binding sites in the membrane during depolarization and enters the cell. It may be that "tubules" seen within the Z lines carry the calcium to the cell interior and the calcium then diffuses longitudinally along the I bands. Hodgkin and Keynes demonstrated that microinjected Ca^{45} did not diffuse through the sarcoplasm and that intracellular calcium was in bound form. An alternate explanation to calcium diffusion would be that influx of calcium creates a chain reaction by releasing calcium held in metastable form along the "tubules" and within the cell.

Despite the recent rapid advances in electrophysiology and microtechniques, only vague outlines of the mechanism of ion transport and muscle contraction are becoming apparent today. Much work remains before a clear understanding of cellular events is at hand.

SUMMARY

1) The muscle cell membrane possesses properties of selective permeability, excitability, and conductivity.

2) The sarcolemma exhibits a resting potential which governs its permeability to ions. This potential in turn is due to the ionic gradients.

3) The high intracellular potassium concentration and the low intracellular sodium concentration is explained by the relative impermeability of the membrane to sodium and the active extrusion of sodium from the cell (sodium pump).

4) The action potential occurs when the cell membrane becomes highly permeable to sodium ions, causing a positive sodium inward current. Repolarization follows with a positive outward current of potassium ions and a decreasing membrane permeability to sodium. The final restoration of the resting potential occurs with the active extrusion of sodium.

5) Several theories have been postulated to relate membrane depolarization to the initiation of muscle contraction.

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A STUDY ON HYPERCOAGULABILTIY USING HEPARIN LOADING TEST

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During recent years increasing attention has been paid to the role of hypercoagulability in the pathogenesis of arteriosclerosis and intravascular thrombosis. Since there are several reviews and publications on this extensive research field, we shall not discuss the literature in detail.

In order to reveal "hypercoagulability" many workers introduced several methods. We suggested[17,18] "heparin loading test" for this purpose. We are going to discuss in this paper the results which we obtained using this method in the cases of arteriosclerosis, ischaemic-heart disease, myocardial infarction, essential hypertension and thrombophlebitis.

METHODS AND MATERIAL

The coagulation time was measured with the method of Lee and White[3], one-stage prothrombin time with the method of Quick[13], antithrombin time with the method of Studer[15] and Winterstein, and heparin clotting time with the method of Rosenthal[14] were performed. The thromboplastin generation test was used according to Biggs[2] and Douglas.

The heparin loading test according to Ulutin[17,18] and Sestakof was performed as follows: 100 mg. heparin† was given intravenously before, and 15, 30, 60, 90, 120, 180, and 240 minutes later venous blood was obtained and thromboplastin formation was followed by means of thromboplastin generation test of Biggs and Douglas. We used the serum after 4 hours of clotting incubated in 37° C waterbath.

In the simplified heparin loading test blood samples were taken before heparin injection, 20 and 60 minutes after.

Cholesterol and total lipid were determined and lipidogram was performed. These experiments were made on ten normal subjects and twenty-two patients.

RESULTS

Antithrombin time shortened in 7 cases out of 12, and in 5 cases out of 14 heparin clotting time diminished. We noticed also that in 14 cases out of 22 the thromboplastin curve was higher than the normal with the thromboplastin generation test. With heparin loading test we observed the following results; in 7 out of 10 normal subjects the antithromboplastic effect of heparin continued 90 - 180 minutes (Fig. I). In three subjects out of ten the mild antithromboplastic effect lasted 60 - 90 minutes.

In 16 cases out of 22 the antithromboplastic effect of heparin lasted 15 - 30 minutes and at 60 - 90 minutes we obtained normal curve (Fig. 2).

However, in six cases antithromboplastic effect of heparin could not be found in the blood even 15 minutes after the injections (Fig. 3).

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†Liquemin "Roche" and Heparin Vitrum were used.

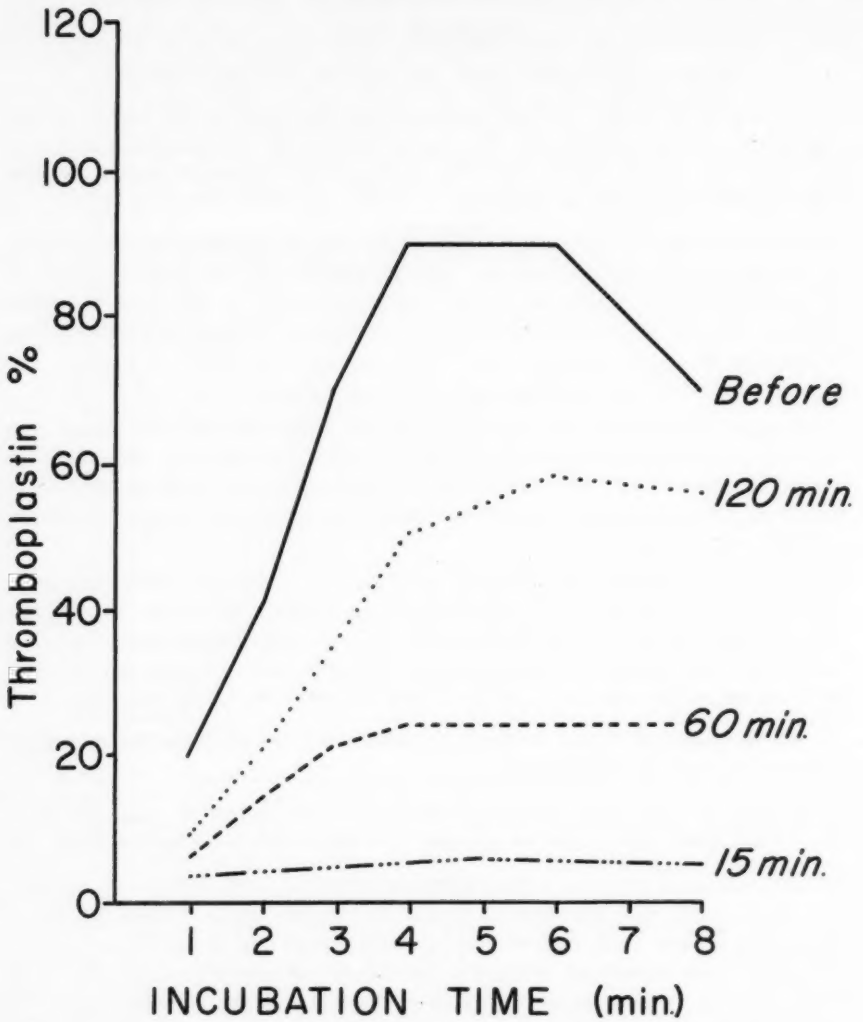

Figure 1

DISCUSSION

Biggs[3], Douglas and MacFarlane in 1953 have shown that heparin inhibits the formation of blood thromboplastin. In 1954 MacMillan[6] and Brown confirmed the antithromboplastic effect of heparin, and they also observed that intravenous heparin causes a defect in serum fraction. Douglas[4] in 1956 showed that in the blood which was taken after injecting heparin AHG, Factor V and prothrombin were not consumed.

In 1957 we confirmed[17] that in the blood taken after heparin injections the generation of thromboplastin was inhibited, and we pointed out that the continuation of

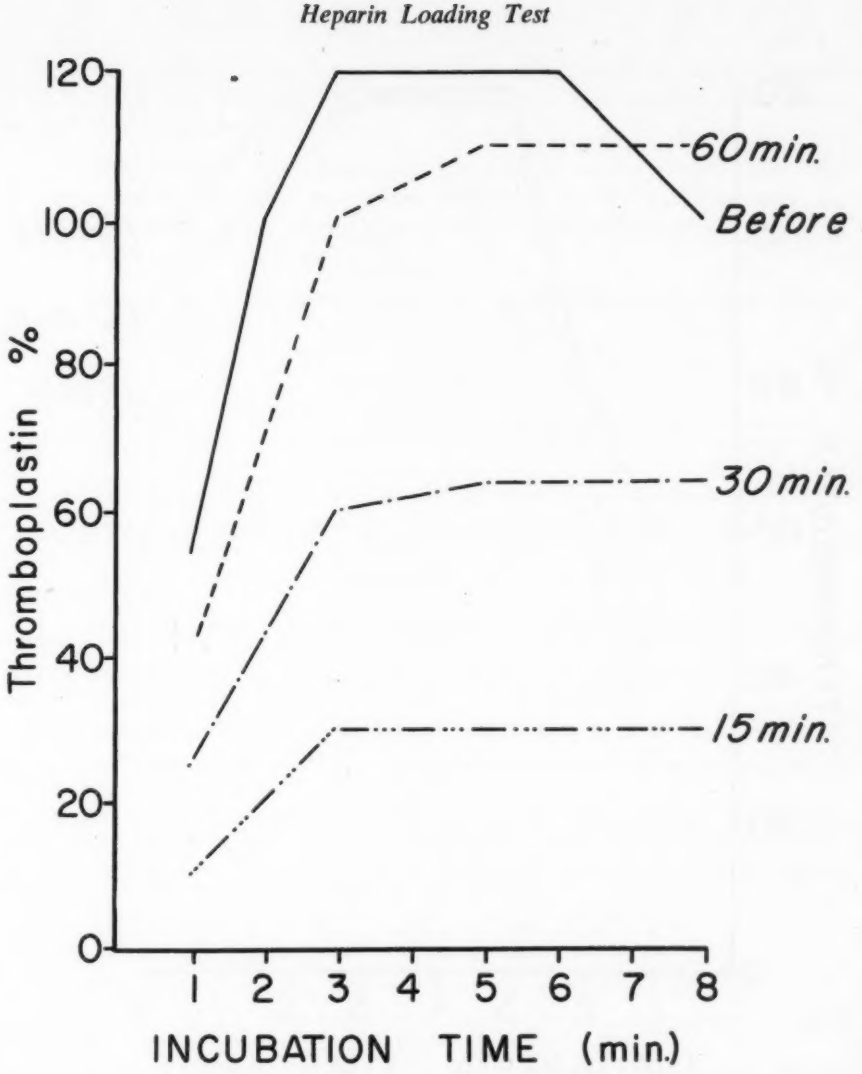

Figure 2

antithromboplastic effect of heparin was different in normal persons and in some patients. In several patients having arteriosclerosis, ischaemic heart disease and thromboplebitis the antithromboplastic effect of heparin lasted for a short time or did not appear at all. In other words, heparin was either consumed or neutralized rapidly.

In cases showing hypercoagulability, according to the heparin loading test, after three or six months of strict low-fat diet and anticoagulant treatment, hypercoagulability decreased or became normal[16] (50 mgs heparin twice weekly intravenously). In

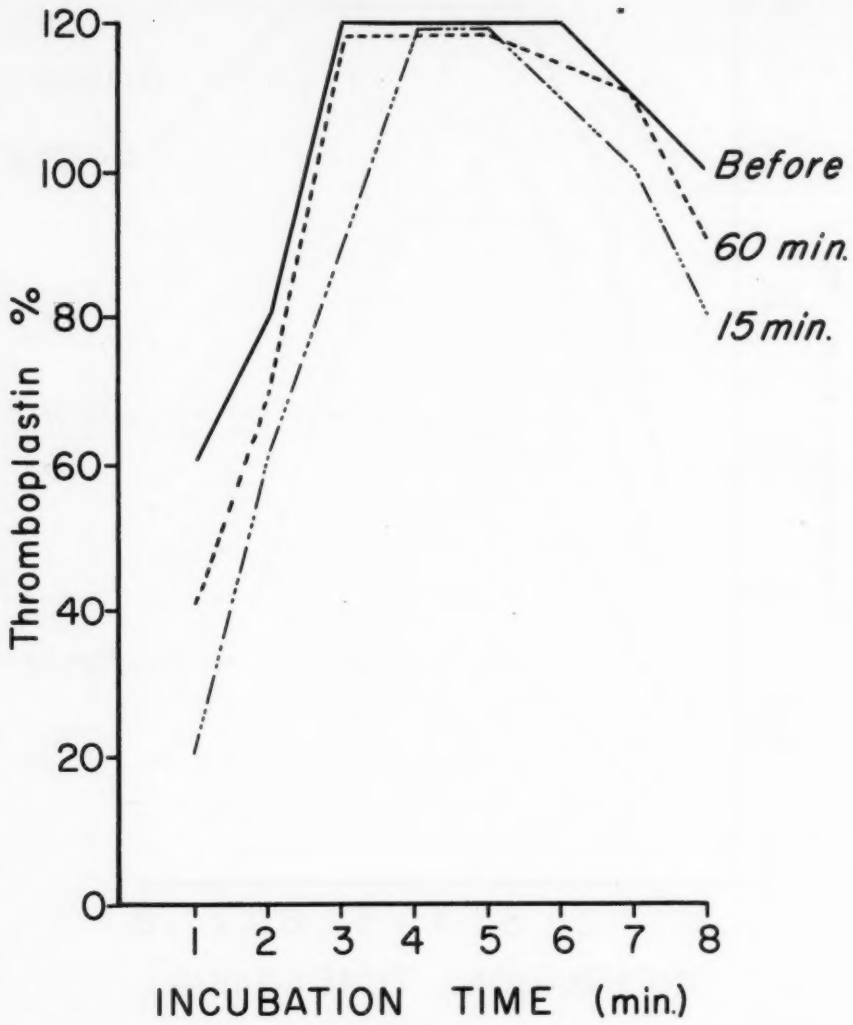

Figure 3

these so-called hypercoagulability cases cholesterol and blood lipid level increased. Lipidogram showed a decrease in A lipids and an increase in B lipids.

In 1957 McDonald[7,8] and Edgill in such cases obtained a shortening of Stypven time, an increase of platelet stickiness and a higher thromboplastin curve in thromboplastin generation test. Authors dwell upon the relation between the increase of cholesterol level and platelet-stickiness.

Heparin Loading Test

In 1957 Kirk[5] and others and in 1958 Berenson[1] have shown that vascular endothelium is lined with a heparin-like mucopolysaccharides, and this substance has an anticoagulant activity.

In 1958 O'Brien[11,12] showed that heparin influenced the Christmas factor. According to author, heparin has affinity for platelets, the Christmas factor and lipoproteins. On the other hand Mustard[9,10] showed that in patients with arteriosclerosis activity of the Christmas factor was increased.

In our opinion there is a close relation between the results of the heparin loading test and recent findings in the literature.

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HYPOGLYCEMIA

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Hypoglycemia, a state often suspected clinically, but one which must be proved chemically, is a potentially serious condition and may pose a threat to life or cerebral function. The purpose of this paper is to review the clinical features suggesting hypoglycemia, the etiologic factors, mechanisms of pathogenesis and the differential diagnosis. Non-specific diagnosis is not difficult and is provided by a low blood sugar value. Specific diagnosis, however, is vital in order to institute rational management. In taking the history particular care must be taken in establishing the relationship of symptoms to time of day, meals, exercise, emotional upsets and environmental changes. Symptoms may have presented with suddenness or may have been insidious in onset. There is frequently an episodic occurrence of severe or mild symptoms. Convulsions and loss of consciousness is common. Profound personality changes can occur.[1] Manifestations of the hypoglycemic state may include any of the following: weakness, excessive fatigue, nervousness, anxiety, palpitations, tremors, sweating, nausea, syncope, numbness of the face, epigastric pain, tachycardia, faintness, headaches, hunger, stupor, drowsiness, pallor, restlessness, chilliness and flushing. The patient is often obese because he has learned food will relieve the distressing symptoms. With prolonged hypoglycemia irritability and confusion may result. Aphasia, diplopia, nystagmus, mania, amnesia, Babinski's sign, disorientation, twitching, convulsions without localization and eventual death may ensue.[1] Often the symptoms are primarily those of adrenergic activity. They may, however, be primarily symptoms of derangement of the central nervous system. The explanation proposed for the former is that hypoglycemia is a stimulus for the release of epinephrine which provokes hepatic glycogenolysis. When there is a rapid fall in blood sugar, symptoms are usually those of adrenergic activity. Glucose constitutes the principal source of energy for the brain. Thus a slow decline in blood sugar is apt to produce cerebral symptoms which, with prolonged hypoglycemia, may present quite a bizarre picture.[2] Nocturnal hypoglycemia may be manifested only by difficulty in awakening. In McQuarrie's type of hypoglycemia of infancy and childhood severe brain damage may occur due to hypoglycemia. After a few years the hypoglycemic state usually disappears leaving a brain damaged youngster of inapparent etiology. The diagnosis can seldom be made unless it is made early in the disease.[3] During attacks of hypoglycemia the EEG may show a slow rate which increases upon the administration of glucose.[4]

If hypoglycemia is suspected the degree must be found at the time of symptoms and relief of attacks upon administration of intravenous glucose must be demonstrated to prove the diagnosis.[5] There is no sharp line of demarcation between normoglycemia and hypoglycemia but it is generally accepted that fasting levels below 50 milligrams percent, if consistently and repeatedly present, represent a significant abberation in carbohydrate metabolism.[4] Almost without exception, except in earliest infancy, patients have symptoms with a blood sugar level less than 50 milligrams percent.[4] Often the diagnosis is missed and the symptoms are ascribed to various other diseases. Fabrykant[6] has stressed the fact that there is not necessarily co-relation between various symptoms and exact level of blood sugar since the brain reflects

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change in blood sugar levels as well as low blood sugar itself. Many persons suffering from attacks of hypoglycemia are labelled psychoneurotics. The diagnosis of psychoneurosis depends on the establishment of positive evidences of neurotic personality structure.[7]

An excellent classification of the hypoglycemic states is that of J. W. Conn.[2] It is included here in revised form in the light of some more recent information on the subject.

I. Organic

A. *Hyperinsulinism:*
   1. Single pancreatic islet cell adenoma
   2. Multiple pancreatic islet cell adenomata
   3. Aberrant pancreatic islet cell adenoma
   4. Pancreatic adenoma associated with other endocrine gland adenomata
   5. Pancreatic islet cell "suspiciously malignant" adenoma (localized)
   6. Pancreatic islet cell carcinoma with metastases
   7. Generalized hypertrophy and hyperplasia of islet cells
   8. Deficiency of islet alpha cells

B. *Hepatic disease:*
   1. Toxic hepatitis
   2. Ascending, infectious cholangiolitis
   3. Diffuse carcinomatosis
   4. Fatty degeneration
   5. Glycogen storage disease of the liver
   6. Laennec's cirrhosis
   7. Infectious hepatitis
   8. Chronic passive congestion

C. *Anterior pituitary hypofunction:*
   1. Destructive lesions; chromophobe tumors, cysts, etc.
   2. Atrophy and degeneration, Simmond's Disease
   3. Surgical hypophysectomy
   4. Pituitary myxedema
      a. thyroid hypofunction secondary to pituitary failure
      b. pituitary failure secondary to myxedema
   5. Severe inanition
   6. Post-operative hypoglycemia

D. *Adrenal-cortical hypofunction:*
   1. Idiopathic cortical atrophy
   2. Destructive infectious granulomata
   3. Destructive neoplasm
   4. Congenital adrenal hyperplasia

E. Fibromata and sarcomata

F. Central nervous system lesions

G. Primary thyroid disease

H. Galactosemia

I. Malabsorption syndrome

II. Functional

A. Hyperinsulinism — imbalance of autonomic nervous system, "hypoglycemic fatigue", nervous hypoglycemia, functional hypoglycemia, reactive hypoglycemia.

B. Alimentary hypoglycemia (rapid intestinal absorption after gastro-enterostomy)

C. Hyperinsulinism of infancy (Staub-Traugott phenomenon)

D. Idiopathic spontaneous hypoglycemia of infancy:
   a. Leucine insensitive
   b. Leucine sensitive

E. Renal glycosuria

F. Lactation

G. Severe continuous muscular work and starvation

III. Factitious

Conn would exclude the concept of "relative hypoglycemia" which confuses diagnosis and treatment. This concept is applied to patients with vague emotional or gastrointestinal symptoms whose blood sugar levels either when symptomatic or during glucose tolerance tests remain essentially normal. It is implied that such individuals are unusually sensitive to minor depressions of blood sugar values. In all patients with unequivocal symptom-producing hypoglycemia, blood sugar values of less than 55 milligrams perecent (Folin-Wu) or 40 milligrams percent (Somogyi-Nelson) can be demonstrated repeatedly. Spontaneous hypoglycemia and hyperinsulinism are not synonymous: the former is a generic term including all conditions in which the blood sugar falls to abnormally low levels. Hyperinsulinism is used when there is evidence of an absolute rise in insulin.[2]

I. Organic

A. Hyperinsulinism:

About 400 cases of functioning islet cell tumors have been reported.[8] About 70 percent are benign; 10 percent are malignant with metastases; 20 percent are microscopically malignant but without metastases. Diffuse hyperplasia and hypertrophy is based on a normal diameter of 20-150μ. Islet cell adenomata range from 0.1-15.0 cm. in diameter but most commonly range from 0.5-2.0 cm. in diameter.[9] They are encapsulated, purplish and firmer than pancreatic tissue, and may be found in the surface or deep within the pancreas. They occur more often in the tail of the pancreas and 12 percent of patients have multiple adenomata. They are occassionally extra-pancreatic, usually in the stomach or in a Meckel's diverticulum. If malignant, metastasis

is first to the regional nodes, then to the liver. The mechanism of hypoglycemia is the autonomous production of insulin by the beta cell tumor. Perhaps not a true hyperinsulinism but certainly a related condition would be the cases reported by McQuarrie[3] of deficiency of pancreatic alpha cells leading to a relative excess of beta cells.

B. Hepatic:

Mellinkoff and Tumulty[10] have emphasized the fact that hepatic hypoglycemia is not uncommon. It occurs as a consequence of impaired glycogenesis, glycogenolysis or gluconeogenesis and serves to emphasize the role of the liver as an organ of homeostasis. In glycogen storage disease, of which 5 different types are now biochemically recognizeable[11], glycogenolysis is impaired.[12] In type I, glycogen storage disease of the liver and kidneys (von Gierke's disease), there is very often hypoglycemia due to the enzymatic defect resulting in a failure of glycogenolysis. In type III, diffuse glycogenosis with hepatic cirrhosis, there is a defect in the debrancher enzyme but hypoglycemia has rarely been reported.

C. & D. Anterior pituitary and adrenal hypofunction:

Both conditions appear to produce hypoglycemia as a result of decreased gluconeogenesis. The roles of somatotropin and the diabetogenic factors are still nebulous. However, decreased ACTH due to pituitary deficiency probably is the important cause here, although decreased thyroid function may play a role. Adrenal cortical insufficiency produces a deficit in gluconeogenesis. Adrenal masculinizing tumor can produce hypoglycemia — probably by a deficiency of glucocorticoids due to the biosynthetic defect which leads to the production of abnormal amounts of 17 keto-steroids.

E. Fibromata and sarcomata:

Silvis and Simon[13] found in a review of the literature 5 cases of organic hyperinsulinism which clinically could not be differentiated from pancreatic adenomata but which proved eventually to be due to fibromata, a spindle cell sarcoma, other sarcomata and a peritoneal pseudomyxoma. They reported a case in which a benign pleural fibroma produced this type of disturbance. Some of the above tumors were assayed for insulin and none was found. They proposed two theories to explain how such tumors might produce the symptoms seen with functioning islet cell adenomata: 1) the tumors might absorb a large amount of glucose, 2) the common denominator of all 6 reported cases was displacement of viscera by the tumor, hence, perhaps they produce a pressure effect in the pancreas.

F. Central nervous system lesions:

Tumors and other diseases involving the hypothalamus may be responsible for hypoglycemia. Certain brain tumors have been reported as being associated with hypoglycemia.

G. Hypothyroidism:

Cretins have long been known, upon occasion, to experience hypoglycemia[3,14]. Also thyroid deficiency may be responsible for reduction of intestinal absorption of sugar probably because thyroxin influences the rate of absorption by regulating phosphorylation.[14]

H. Glactosemia:

The enzymatic inability to utilize galactose results in a hypergalactosemia which brings about a depression of blood glucose.[11]

I. Malabsorptive states:

Chronic diarrheas, such as the celiac syndrome, are at times a precipitating cause of hypoglycemia (as in the case when starvation is superimposed) but rarely a primary cause.[14]

II. Functional:

A. Hyperinsulinism:

Hyperinsulinism in the absence of an organic lesion has been thought to be due to autonomic nervous system dysfunction. It has been suggested that in some individuals a high blood sugar rapidly brings about increased insulin production which causes a rapid fall in blood sugar level. This has been called a psychosomatic disorder and functional hyperinsulinism is the most common form of hypoglycemia.[2] Here there is abnormal response of functionally hyperactive islands. Symptoms are usually attributable to the effects of epinephrine which is released after the fall in blood sugar.

B. Alimentary hypoglycemia:

In alimentary hyperinsulinism there is a normal response to intense stimulation. It is to be differentiated from the "dumping syndrome" which occurs sooner in the post prandial period and believed due to hyperosmolarity of intestinal content.[2] Alimentary hyperinsulinism is second only to functional hyperinsulinism in frequency. Rapid sugar absorption quickly produces a high blood sugar level. Hypoglycemia is produced by much the same mechanism as functional hyperinsulinism but here the stimulus abnormally increased and the function of the islet cells normal. It is seen whenever sugar can enter the small intestine rapidly in high concentration such as after gastroenterostomy.

C. Hyperinsulinism of infancy (Staub-Traugott phenomenon):

There is some evidence[3] that spontaneous hypoglycemia may occur in children and adults as a result of over stimulation of normal beta cells by frequent and excessive ingestion of carbohydrate.

D. Idiopathic spontaneous hypoglycemia of infancy:
This entity is discussed later, however, this is to be separated from the

cases McQuarrie and his associates[12] have recently described in which they presented 3 cases of spontaneous hypoglycemia in infancy which were thought due to glucagon deficiency. All occurred in one family and each of the children began having symptoms, including convulsions, during the first year of life. When partial pancreatectomy was performed it was found that the islets were devoid or nearly devoid of alpha cells (vide supra).

E. Renal glycosuria:

This will be discussed later. True renal glycosuria can produce hypoglycemia as it occurs in the fasting state, progressively lowering the blood sugar.[15]

F. & G. Lactation, severe continuous musclar work and starvation:

Lactation and severe continuous musclar work may cause hypoglycemia by progressively lowering blood sugar and possibly by diminution of glycogen stores. Fabrykant et al[16] feel unusual sensitivity to insulin may develop as a result of inanition. This was observed in diabetics treated with starvation diets and was ascribed to glycogen deficiency. In a case described by Joslin a reaction followed the administration of 1 unit of insulin. Starvation may be a common cause of hypoglycemia in infants and children.[16,14] It is frequently found in the first few days of life and in babies prematurely born but the hypoglycemia in early infancy is very rarely associated with symptoms.[18] This is noteworthy in the case of the baby of the diabetic mother who is often profoundly hypoglycemic but rarely is symptomatic on that basis. Adrenal hemorrhage of the newborn may cause a sympotmatic hypoglycemia but the condition is often fatal for other reasons.

III. Factitious:

The injection of insulin by the patient or physician must, of course, be included in a discussion of hypoglycemia. This cause is excluded by the term spontaneous, however, it is necessary to consider this possibility in the differential diagnosis. Hypoglycemic reactions due to the Sulfonylurea drugs have been reported.[19] Innocently erroneous insulin dosage must be considered in the diabetic who cannot read his syringe well because of retinopathy or cataracts.

LABORATORY AIDES TO THE DIFFERENTIAL DIAGNOSIS OF HYPOGLYCEMIA

The presence of hypoglycemia is determined by measuring the blood glucose concentration. The finding of abnormally low blood glucose establishes only the fact of hypoglycemia. However, the relationship of such a finding to certain events is most important in arriving at a specific diagnosis. The glucagon test is in the experimental stage[13] and is used mainly in the diagnosis of von Gierke's disease. The tests of liver function are used frequently. The 6 hour glucose tolerance test as described by Harris[20] in 1936, is performed by giving the patient 1.75 grams of glucose per kilogram of body weight in 250 cc. of water when in the fasting state. A blood sugar is drawn before the test and at hourly intervals for 6 hours. A

positive diagnosis of hypoglycemia is made if one of the samples contains less than 70 milligrams percent of glucose. When the test is positive at least 2 specimens usually contain less than 70 milligrams percent.[20] A modification of this test by Abrahamson[21] consists in the patient having dinner at 6 p.m. and being furnished with 100 grams of glucose (for adults) in 250 cc. of water with lemon juice. The patient sets his alarm for 4 a.m., drinks the solution and goes back to sleep. At 9 a.m. the blood sugar is drawn. If there is less than 70 milligrams percent, a diagnosis of hypoglycemia can be made. The glucose tolerance test as described by Albright et al.[22] is used to study the reaction to alimentary hyperglycemia and its chief value occurs when an abnormal response is elicited, i.e., unresponsiveness to hyperglycemia (decreased sugar tolerance.) Hyperglycemia resistence (increased sugar tolerance) can be looked for but the abnormality which such a finding suggests can be brought out better, according to these workers, by another method. Albright et al. felt it better to select a method which will bring out a weakness in a compensatory mechanism rather than one which elicits an increased aptitude. However, great use is made of this test. The insulin tolerance test according to Albright et al.[22] has two purposes: 1) to measure the sensitivity of the blood glucose level to insulin, and 2) to ascertain degree of responsiveness to insulin induced hypoglycemia. Insulin is given intravenously. Normally the serum glucose falls to about 50% of the fasting level at the 20 to 30 minute sample. Once the level of 50 milligrams percent has been obtained the mechanism for responding to hypoglycemia should be stimulated and the blood sugar should return to the fasting value in 60 to 100 minutes. There are two reactions to look for: 1) insulin resistence, 2) hypoglycemia unresponsiveness (an absent or delayed rise). The glucose — insulin tolerance test[22] in which glucose is given orally and insulin intravenously has one purpose; to determine if the patient is unresponsive to hyperglycemia. It is not used in the diagnosis of hypoglycemic states. Other laboratory methods will be discussed under appropriate headings.

Conn[2] advocates that in establishing the specific etiology of hypoglycemia it first be determined if the hypoglycemia is 1) fasting, 2) stimulative or 3) both fasting and stimulative. He would divide them as follows:

1. Fasting 1) hepatic, 2) anterior pituitary hypofunction, 3) adrenal-cortical hypofunction, 4) CNS lesions, 5) fibroma and sarcoma, 6) severe renal glycosuria.

2. Stimulative: (normal blood sugar until after carbohydrate imbibition) 1) functional, 2) alimentary, 3) hyperinsulinism of infancy (Staub-Traugott phenomenon).

3. Combined: 1) organic hyperinsulinism, 2) idiopathic spontaneous hypoglycemia of infancy, 3) factitious.

One should first investigate adrenal insufficiency, pituitary insufficiency, CNS disease, alimentary hypoglycemia, lactation, renal glycosuria and factitious hyperinsulinism. If the hypoglycemia is due to adrenal insufficiency, there will be a fasting type of hypoglycemia; i.e., the symptoms will appear upon deprivation of food, be present in the pre-breakfast zone and after exercise. There should be physical and

laboratory evidence of Addison's disease, adrenal insufficiency, or virilism to make the diagnosis. As confirmation the insulin tolerance test should show hypoglycemia unresponsiveness. If hypoglycemia is due to pituitary insufficiency there will be other findings, e.g., x-ray evidence of pituitary tumor, visual field deficits, evidence of decreased thyroid activity or decreased growth.[9] An abnormal insulin tolerance test confirms hypoglycemia unresponsiveness due to diminished ACTH production. In primary hypothyroidism the insulin tolerance test shows diminished insulin responsiveness rather than increased.[22] In the case of central nervous system disease, neurological signs are usually present.

Alimentary hypoglycemia is purely stimultive, occurring only post-prandially. There will be an abdominal surgical scar and a history of abdominal surgery. The symptoms usually follow the stimulus of meals and tend to be of the same pattern and severity. This can be diagnosed with the knowledge of the type of operation performed and proof that the hypoglycemia is purely stimulative.

If lactation is suspected as being the cause of hypoglycemia a remission at the cessation of lactation may be expected. Then the patient will be seen to regain a normal fasting blood sugar concentration.

In true renal glycosuria[15] there should be a fasting blood sugar below 100 milligrams percent and a normal or flat glucose tolerance curve. Glucose should be present in every specimen of urine tested. In pseudo-renal glycosuria[15] the glycosuria disappears when the blood sugar level falls toward 100 milligrams percent. A provocative test (fasting or insulin tolerance test) may be necessary to demonstrate this and no suagr is found in the fasting urine. It is distinguished from alimentary glycosuria since the latter occurs only when blood sugar level is high.

A history of insulin administration can often be obtained in cases of factitious hyperinsulinism.[1] Symptoms vary depending on whether the reaction is due to crystalline or a depot insulin. With crystalline insulin there will be a sudden onset of symptoms usually with extreme weakness 15 to 20 minutes after the injection of insulin. Depot insulin produces a gradual and insidious onset of symptoms, manifasted chiefly by headaches and changes of mood. The reaction occurs approximately 4 hours or 8 to 24 hours after modified insulin. If PZI is the causitive agent one must beware of a recurrence of symptoms 3 to 4 hours after successful treatment by glucose administration. Nocturnal hypoglycemia, as previously mentioned, is manifested chiefly by difficulty in awakening.

The differential diagnosis of the hypoglycemic state usually centers about the differentiation of functional hyperinsulinism, organic hyperinsulinism or hepatic hypoglycemia. According to Conn[2] 80% of cases will be of these three types.

ORGANIC HYPERINSULINISM

The classical symptoms of organic hyperinsulinism are recurrent, and usually begin insidiously. Periodicity of symptomatology is present and symptoms tend to become more frequent and severe. Occasionally there may be sudden convulsive seizure and loss of consciousness. A. O. Whipple established a triad of characteristics for

the disease entity with few exceptions, the exceptions will be discussed. The triad[9] consists of: 1) the attacks come on characteristically in the early morning during the fasting period before breakfast or after severe mental or physical effort when the hypoglycemia is present, 2) during attacks or after a fast of 12 to 24 hours the blood sugar levels are always below 50 milligrams percent, 3) symptoms are completely relieved by administration of glucose by mouth or intravenously. The presence of this triad is a prime requisite for the diagnosis of islet cell tumors.[9] However, in 1944, Whipple reported himself wrong in the diagnosis in one out of five patients fitting this triad. In 1952, Whipple[8] reported 87% diagnostic accuracy obtained with these criteria.

Conn[5] has stated that 1) exploration is essential if there is a low blood sugar during symptoms and there is relief of symptoms upon administration of glucose, 2) there can be proven no extrapancreatic reason for the hypoglycemia, 3) if there is a fasting blood sugar repeatedly below 50 milligrams percent with adequate diet and below 40 milligrams percent on carbohydrate restriction, and 4) if the symptoms are severe, progressive and are of short duration in a person passed middle age. With these criteria 83% will be found to have a pancreatic tumor.[5] In functional hyperinsulinism the 15 hour fasting blood sugar is normal, in organic it is low. Hepatogenic hypoglycemia has a fasting blood sugar that is also low. The glucose tolerance test is useful in differentiating these entities; in functional hyperinsulinism the fasting blood sugar is normal and there is a rapid fall to low blood sugar levels in 2 to 4 hours. In organic hyperinsulinism the fasting blood sugar is low and the glucose tolerance test results in a low curve with a sharp fall in 2 to 5 hours. In hepatic hypoglycemia there is a low fasting blood sugar and a hyperglycemic curve and a fall to hypoglycemia in 4 to 7 hours. The organic type is made worse by exercise or fasting; as is the hepatogenic type. The functional type is purely stimulative and never occurs before breakfast but occurs after meals and is seen mainly in persons under emotional tension and in environmental upsets.

McQuarrie's cases will be discussed at this point and should be distinguished from the Staub-Traugott phenomenon which is a stimulative type of hypoglycemia due to excessive sugar intake for a prolonged period of time.[2] McQuarrie did not postulate alpha cell deficiency for idiopathic spontaneous hypoglycemia of infancy and childhood. The symptoms are similar to those of tumor in the adult. There is a low fasting blood sugar. The glucose tolerance test shows a sharp fall in blood sugar placing this entity in the combined fasting and stimulative group. The condition subsides with age. The reasons ordinarily given for operation in suspected tumor of islet cells are: 1) there is a 9% incidence of malignancy; 2) obesity is common making eventual surgery more difficult; 3) the danger to the central nervous system of uncontrolled hypoglycemia. However, because this condition subsides and because no carcinoma has been reported under age 18 years[2] and since it shows good response to medical treatment, surgery is not advocated in infants and young children. McQuarrie feels that one cannot differentiate this condition from an islet cell tumor but there is a highly favorable clinical response with normoglycemic levels and cessation of symptoms upon administration of ACTH which he considers diagnostic of this entity. The diagnostic value of the response to ACTH is limited by the fact that patients

with pancreatic tumor, and some with von Gierke's disease, also respond favorably. However, pancreatic tumors are extremely rare in this age group and McQuarrie felt identical findings in an older group would suggest islet cell tumor. It has recently been shown[11] that the adenocorticoid steroids can replace ACTH in the treatment of this entity. McQuarrie found that 25 of a series of 40 cases of infantile hypoglycemia fell into this category (idiopathic). Recently British workers[23,24] have found this type of case to fall into two subdivisions which are designated as either leucine sensitive or leucine insensitive. Leucine sensitive patients do not become hypoglycemic when fed a leucine free diet. The casein tolerance test may be used as a provocative test to determine if leucine-rich casein will produce the characteristically abnormal hypoglycemic response. Further work on the leucine insensitive group[23] revealed that some of the patients responded in an unexpected fashion to the adrenalin and glucagon tolerance tests with supernormal responses. The circulating insulin level was high normal. Not only did ACTH control the condition but a diminution in urinary catecholamines was found and thought perhaps to be due to mild adrenaline insufficiency which would result in impaired glucose homeostasis.

A paient recently diagnosed at the Henry Ford Hospital (934084) exemplifies many of the features of McQuarrie's disease. The patient, a white female, began having generalized seizures at six months of age. The pregnancy and delivery were normal save for a maternal hemorrhage in the fourth month. After age six months the child experienced numerous convulsive seizures, particularly when infected, but at times without associated fever. During periods of anorexia, with or without apparent infection, she would become "limp" and if feedings were not accepted convulsions would ensue. It was noted that after the afternoon nap the baby was difficult to awaken and would become alert only after a food was given. Convulsions also occurred during the night. The patient was often drenched by perspiration, especially when "limp." She was most alert after eating and the symptoms rarly occurred in less than four hours after eating. The patient was always quite placid and at 19 months had not begun to walk or talk. A grandmother had diabetes mellitus and an older male sibling had febrile seizures as an infant. The paternal grandfather had to have his meals at carefully timed intervals to prevent irritability. The patient had previously been hospitalized but glucose metabolism had not been investigated. Electroencephalograms had been normal as were determinations of the serum proteins, calcium and phosphorus. Phenylalinine metabalites were absent from the urine and there were no reducing substances in the urine. An x-ray of the skull was normal. Numerous anti-epileptic medications had been prescribed without success.

At 19 months of age a blood glucose was determined during a convulsion and the value was 36 mg. %. She was then rehospitalized. The patient was obese, weighing 26 lbs. She did not walk or talk and was rather inert and hypotonic. Other than the placidity the physical examination was normal. The protein bound iodine, serum protein electrophoresis, bone age, serum electrolytes and tests of liver function were normal. An EEG again was normal. Repeated oral glucose tolerance tests were performed and the results were fasting blood sugars that ranged from 30 to 90 mg. %. The highest values appeared at ½ to 1½ hours (172-234 mg. %) and the lowest values were 20 mg. % at 3½ and 39 mg. % at 6½ hours. A casein tolerance test was attempted (1.5gm. per kg.) but the patient vomited the gelatinous mixture. The bloods, however, were drawn and the glucose ranged from 21 mg. % (before test material) to 54 mg. % at 1 hour and 32mg. % at 4½ hours. Epinephrine raised a fasting glucose of 50 mg. % to 108 mg. % at 15 minutes.

Prednisone was then administered (5 mg. every 8 hours) and convulsions disappeared. Blood glucose was then determined at various times relative to meals and these were within normal limits. The child was also placed on 20% protein feedings every four hours during the day. One month after instituting the steroid the child was walking and had begun to talk. No seizures had occurred. There were occassional episodes of limpness and perspiration — more easily alleviated by meals than before. Triamcinalone was then used to obtain gluconeogenesis with less danger of steroid side-effects. Three months later the child was romping about the home and had remained free from seizures. There was occassional excessive perspiration. The mother had been furnished with glucagon for injection in the event of alarming symptoms but the need had not arisen.

FUNCTIONAL HYPERINSULINISM

Functional hyperinsulinism shows no tendency to progress in severity and there are periods of complete remission, usually during a vacation or change in environment.[5]

This is the most common cause of hypoglycemic attacks in the adult. The illness usually occurs in a person subject to anxiety or in a person with emotional tension. Clinically the attacks occur after meals and usually consist of symptoms of adrenergic activity. Sugar alleviates the attacks but may cause another episode. One to three hours after a meal the blood sugar is in the range of 20 to 50 milligrams percent. The fasting blood sugar level is characteristically normal, it is low in the organic and hepatogenic types. A glucose tolerance test will show a precipitous fall in blood sugar. An insulin tolerance test or epinephrine or glucagon test will show normal glycogen reserves. This type of hypoglycemia is purely stimulative and never occurs in the fasting state.[2] The symptoms of hyper-adrenalism last about 15 to 20 minutes, occur 2 to 4 hours after a meal and loss of consciousness or convulsions are said never to occur. This is not a progressive disease but the symptom picture varies with circumstances. The glucose tolerance test is pathognomonic[2] with a normal fasting blood sugar and then a rapid and profound fall in blood sugar. If doubt remains as to the validity of the assumption of a normal fasting blood sugar, a 36 hour fast may be used[2] or one may give a high protein, high fat, low carbohydrate diet for 3 days, then fast the patient for 36 hours. The patient should then be exercised to finally differentiate the condition from organic hyperinsulinism for such provocation may prove the illness also belongs in the fasting group. If a diagnosis of functional hyperinsulinism is made on the above criteria including a characteristic glucose tolerance test one probably should also diagnose an anxiety state.[2]

HEPATIC (hepatogenic) HYPOGLYCEMIA

Tumulty and Mellinkoff[10] state that there may not necessarily be overt evidence of liver disease in hepatogenic hypoglycemia. However, when derangement of liver function is responsible for hypoglycemia there is always laboratory evidence of liver dysfuntion. These patients do not tolerate a diet low in carbohydrate. Thus, they resemble the patient with an islet cell tumor. However, there is a striking difference in the glucose tolerance curve for both show a low fasting blood sugar, but in hepatogenic disease there is a hyperglycemic curve with a fall to hypoglycemic levels 4 to 7 hours later. This is not changed by preparation with a high carbohydrate diet as is sometimes the case with pancreatic tumors. If a hyperglycemic response in the presence of a pancreatic tumor is obtained, it is due to suppression of normal beta cell activity by the functional nature of the tumor.[2] Testing should, if possible, always be done after the patient has received a diet containing 15% protein, and 50% carbohydrate for 3 days. With liver disease there is constantly a hyperglycemic, hypoglycemic curve. If the cause of hypoglycemia is viral hepatitis there will be improvement in the hypoglycemic state correlating well with the recovery of liver function. Hepatogenic hypoglycemia is often called "hepatic coma" and should be suspected when a person with liver disease becomes comatose. It should be added parenthetically that hepatic coma is a consequence of other mechanisms as well. The characteristic glucose tolerance test in addition to laboratory evidence of other liver function derangement makes the diagnosis.[5] Gross states that a normal epinephrine test producing hyperglycemia eliminates the liver as a cause of hypoglycemia.[4]

von GIERKE'S DISEASE

This is a genetically determined metabolic defect and family history should be

minutely investigated. Hepatomegaly is virtually universal. A diagnosis can be made upon biopsy of the liver. Affected persons seldom survive to adulthood and this does not usually enter into the differential diagnosis of adult hypoglycemia. However, recently van Creveld[25] restudied two cases that were originally reported in 1928 and 1932. In both the liver was just barely palpable and the patients were healthy and married and had produced offspring. The fasting blood sugars were on the low side of normal and the blood glycogen had remained increased. In one a glucose infusion gave a distinct plateau indicating a difficulty in glycogenesis and this was followed by a rapid decrease to the prior fasting level. In both the epinephrine test was abnormal. Acetone appeared in the urine after the epinephrine test. Van Creveld commented that the glucose levels tended to become normal but biochemical abnormalities could still be detected. He felt that other metabolic pathways had been utilized to handle the problem of glucose homeostasis. The hypoglycemia due to von Gierke's disease can be distinguished from other forms of hepatogenic hypoglycemia by the glucagon test in which 1 to 2 milligrams will elicit no rise in blood sugar. This test is not yet generally used and in many hands the results have been quite equivocal.[12] However, the response to epinephrine gives some information as to the severity of diminished functioning glycogen reserves. Injection of 0.3 milligrams should cause a rise in sugar of 40 to 70 milligrams percent in one hour if there is no liver disease.

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INTERNATIONAL SYMPOSIUM
THE BLOOD PLATELETS

March 17, 18, 19, 1960

The professional staff of the Henry Ford Hospital with the initial advice and support of the Board of Trustees and Dr. Robin C. Buerki, Executive Director of the Henry Ford Hospital, is sponsoring an international symposium on the subject The Blood Platelets. This symposium was held at the Henry Ford Hospital, March 17, 18, and 19, 1960, in the Clinic Auditorium. The program follows and has been prepared with the help of the advisory members:

PROGRAM COMMITTEE

ADVISORY MEMBERS:

Advisory Chairman
Walter H. Seegers

Professor, Chairman of the Department of Physiology and Pharmacology, College of Medicine, Wayne State University, Detroit, Michigan.

C. Lockard Conley

Professor of Medicine School of Medicine, The Johns Hopkins University, Baltimore, Maryland.

Kenneth M. Brinkhous

Professor, Chairman of Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina.

Carl V. Moore

Professor, Chairman of Department of Medicine, College of Medicine, Washington University, St. Louis, Missouri.

LOCAL MEMBERS:

Robert C. Horn, Jr.
Raymond W. Monto
Shirley A. Johnson, *Chairman*
John W. Rebuck

International Symposium

PROGRAM

First Day — Thursday, March 17, 1960

Morning Session

8:50 A.M. Remarks of Welcome

Robin C. Buerki, Executive Director, Henry Ford Hospital

Introduction

Shirley A. Johnson, Henry Ford Hospital, Detroit, Michigan

PLATELETS IN HEMOSTASIS AND THROMBOSIS

Chairman — *Walter H. Seegers* — Wayne State University, Detroit, Michigan

9:00 The blood platelets — past, present and future

Leandro M. Tocantins, Jefferson Medical College, Philadelphia, Pa.

9:10 Physiology of platelets in the microcirculation

George P. Fulton, Boston University Graduate School, Boston, Mass.

PLATELETS IN BLOOD VESSEL INTEGRITY

9:30 Morphology and dynamics of endothelium

Rudolf Altschul, University of Saskatchewan, Saskatoon, Canada

9:45 Capillary resistance and its relation to bleeding

Jenö Kramàr, Creighton University, Omaha, Nebraska

10:00 Studies on hemostasis, in vivo

Walter O. Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

10:20 General Discussion

10:35 Intermission

The Blood Platelets

PLATELET CLUMPING

First Day — Thursday, March 17, 1960

10:50 Viscous metamorphosis of platelets

Alan A. Sharp, United Oxford Hospitals, Oxford, England

11:10 Morphological studies on the mechanism of viscous metamorphosis of platelets

Robert L. Rosenthal, Beth Israel Hospital, New York, New York

11:25 Phase microscopy and the enumeration of blood platelets

Robert Feissly, University of Lausanne, Lausanne, Switzerland

11:45 General Discussion

12:00 Luncehon

Afternoon Session

Chairman — *C. Lockard Conley* — The Johns Hopkins University, Baltimore, Maryland

PLATELET CLUMPING

1:30 P.M. Natural thrombocyte-agglutinating systems: thrombocyte-agglutinating factor (TAg) and thrombin

Kenneth M. Brinkhous and *Reginald G. Mason, Jr.,* University of North Carolina, Chapel Hill, North Carolina

1:50 In vitro platelet clumping and aggregation in relation to metal ions, macromolecules and surfaces

Charles E. Brambel, University of Notre Dame, Notre Dame, Indiana

PHYSIOLOGY AND PATHOLOGY OF HEMOSTASIS

2:05 Multiple factors in hemorrhage

Louis B. Jaques, University of Saskatchewan, Saskatoon, Canada

First Day — Thursday, March 17, 1960

2:20 Designated discussant

Marjorie B. Zucker, Sloan-Kettering Institute for Cancer Research, New York, New York

2:30 General Discussion

2:50 Intermission

ROLE OF PLATELETS IN THROMBOSIS

Chairman — *Robert W. Wissler* — University of Chicago, Chicago, Illinois

3:00 Role of platelets in the formation of a hemostatic plug
Jacques Roskam, University of Liége, Liége, Belgium

3:20 P.M. Dietary lipids and the clotting mechanism with particular reference to lysis

Wilbur A. Thomas, Albany Medical College of Union University, Albany, New York

3:35 The influence of induced atherosclerosis on calcium, phosphorus and sulfate in the arterial wall

Albert J. Boyle, Wayne State University, Detroit, Michigan

3:50 Lipids, platelets and atherosclerosis

James F. Mustard, University of Toronto, Toronto, Canada

4:05 Summary

4:15 General Discussion

Robert W. Wissler, University of Chicago, Chicago, Illinois

First Evening Session

ROUND TABLE

8:00 — 9:30

Platelets in the physiology of hemostasis with particular reference to bleeding time.

Chairman — *Raymond W. Monto* — Henry Ford Hospital, Detroit, Michigan

PARTICIPANTS:

Dr. Spaet — Montefiore Hospital, New York, New York

Dr. Roskam — University of Liége, Liége, Belgium

Dr. Fulton — Boston University Graduate School, Boston, Massachusetts

Dr. Cruz — Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Dr. Jaques — University of Saskatchewan, Saskatoon, Canada

Dr. Sharp — United Oxford Hospitals, Oxford, England

Dr. Lüscher — University of Bern, Bern, Switzerland

Second Day — Friday, March 18, 1960

PHYSIOLOGICAL AND BIOCHEMICAL MECHANISMS OF PLATELETS IN BLOOD COAGULATION

Chairman — *Armand J. Quick* — Marquette University, Milwaukee, Wisconsin

9:00 A.M. Platelet fractions and prothrombin activation

Walter H. Seegers, Wayne State University, Detroit, Michigan

9:20 Chemical synthesis of phospholipids

Erich Baer, University of Toronto, Toronto, Canada

9:35 The platelet lipids: their identification, quantification and behavior in, in vitro, clotting systems

Stanley B. Troup, University of Rochester, Rochester, New York

9:50 The thromboplastic activity of some soybean phospholipids

William J. Williams, Washington University, St. Louis, Missouri

10:05 Biophysical aspects of platelet reaction mechanisms in clotting

Douglas M. Surgenor, Harvard Medical School, Boston, Massachusetts

10:25 The lipids of human erythrocytes and platelets and their effects on thromboplastin formation

Percy Barkhan and *Melvin J. Silver*, Jefferson Medical College, Philadephia, Pennsylvania

10:40 General Discussion

11:00 Intermission

PLATELET FRACTIONS

Chairman — *Shirley A. Johnson* — Henry Ford Hospital, Detroit, Michigan

11:10 The antifibrinolytic activity of platelets

Norma Alkjaersig, Washington University, St. Louis, Missouri

11:30 Studies on platelet factor 4

Erwin Deutsch, University of Vienna, Vienna, Austria

11:50 The nature of the lipoprotein of human platelets and erythrocytes

George Y. Shinowara, New York University, New York, New York

12:10 P.M. General Discussion

12:30 Luncheon

Second Day — Friday, March 18, 1960

Afternoon Session

Chairman — *Kenneth M. Brinkhous* — University of North Carolina, Chapel Hill, North Carolina

METABOLISM OF PLATELETS

2:00 P.M. The breakdown of phospholipids and of other phosphorous compounds during coagulation

Gustav V. R. Born, University of Oxford, Oxford, England

2:20 Metabolic changes in platelets during viscous metamorphosis and clot retraction

Marjorie B. Zucker, Sloan-Kettering Institute for Cancer Research, New York, New York

2:35 The uptake of serotonin by platelets

Herbert Weissbach, National Heart Institute, Bethesda, Maryland

2:50 Microelectrophoretic studies of platelets

Hazel Warren, Louis A. Kazal and *Leandro M. Tocantins,* Jefferson Medical College, Philadelphia, Pennsylvania

3:10 Metabolic aspects of normal and pathological platelets

Rudolf Gross, University of Marburg, Marburg on Lahn, Germany

3:30 Effects of sulfhydryl inhibitors on structural stability of platelets

J. Leo Koppel, Presbyterian-St. Luke's Hospital, Chicago, Illinois

3:45 General Discussion

4:05 Intermission

CLOT RETRACTION OF PLATELETS

4:15 Platelets in clot retraction

C. Lockard Conley, The Johns Hopkins University, Baltimore, Md.

4:30 Retraction activity of the platelets: biochemical background and physiological significance

E. F. Lüscher, University of Bern, Bern, Switzerland

4:50 Time lapse photography of clot retraction

Giorgio Ballerini and *Walter H. Seegers,* University of Ferrara, Ferrara, Italy and Wayne State University, Detroit, Michigan

5:05 General Discussion

International Symposium

Evening Session

6:30 Reception — Statler Hilton Hotel

7:30 Banquet — Chairman: *Robert C. Horn, Jr.*, Henry Ford Hospital, Detroit, Michigan

The mystery of Indo-European expansion

Clark Hopkins, University of Michigan, Ann Arbor, Michigan

Third Day — Saturday, March 19, 1960

Morning Session

THROMBOCYTOPENIC BLEEDING

Chairman — *Maxwell M. Wintrobe* — University of Utah, Salt Lake City, Utah

9:00 A.M. Evaluation of the degree of bleeding in thrombopenic states

Dudley P. Jackson, The Johns Hopkins University, Baltimore, Md.

9:20 Thrombocytopenic bleeding and preservation of platelets

Frank H. Gardner and *Phin Cohen,* Peter Bent Brigham Hospital, Boston, Massachusetts

9:40 Platelets and heparin

Robert C. Hartmann, Vanderbilt University Hospital, Nashville, Tenn.

9:55 General Discussion

10:15 Intermission

ALTERED PLATELET FUNCTION

Chairman — *Leandro Tocantins* — Jefferson Medical College Philadelphia, Pennsylvania

10:25 Specific platelet alterations related to hematological disorders

Shirley A. Johnson, Henry Ford Hospital, Detroit, Michigan

10:45 Electron microscopy and hematocrit of abnormal platelets

John W. Rebuck, Henry Ford Hospital, Detroit, Michigan

11:05 The qualitative platelet diseases

Orhan N. Ulutin, University of Istanbul, Istanbul, Turkey

11:25 Some qualitative aspects of platelet function

Benjamin Alexander, Harvard Medical School, Boston, Massachusetts

11:45 General Discussion

12:00 Noon Luncheon

Third Day — Saturday, March 19, 1960

Afternoon Session

HEMATOPOIETIC ASPECTS AND PHYSICAL PROPERTIES OF PLATELETS

Chairman — *John W. Rebuck* — Henry Ford Hospital, Detroit, Michigan

1:30 P.M. Studies on the origin, production and destruction of platelets

Eugene P. Cronkite, Brookhaven National Laboratory, Upton, L. I., New York

1:45 Biodynamics of thrombopoiesis

Riojun Kinosita, City of Hope Medical Center, Duarte, California

2:00 Electron microscopy of blood platelets

Herbert Braunsteiner, University of Vienna, Vienna, Austria

2:20 Thrombokinetics

Clement A. Finch, University of Washington, Seattle, Washington

2:35 General Discussion

2:55 Intermission

LABELLING, LIFE SPAN AND PRESERVATION

Chairman — *Carl V. Moore* — Washington University, St. Louis, Missouri

International Symposium

3:05 Platelet labelling with radioisotopes and in vivo platelet survival

T. T. Odell, Jr., Oak Ridge National Laboratory, Oak Ridge, Tennessee

3:20 Survival of platelets transfused into thrombocytopenic recipients

Julius R. Krevans, The Johns Hopkins University, Baltimore, Maryland

IMMUNOLOGICAL ASPECTS OF PLATELETS

3:35 Immune reactions of platelets

William J. Harrington, Washington University, St. Louis, Missouri

3:50 Mechanisms of immunogenic platelet damage

Peter A. Miescher and *Fred Gorstein*, New York University, New York, New York

4:05 The identification and isolation of antiplatelet proteins

James L. Tullis, Protein Foundation, Inc., Jamaica Plain, Mass.

Afternoon Session

4:20 Use of the fluorescent antibody technic in the detection of platelet "antibodies"

Charles C. Sprague, Tulane University, New Orleans, Louisiana

4:35 Discussion

4:45 SUMMATION OF SYMPOSIUM

Carl V. Moore, Washington University, St. Louis, Missouri

LIST OF PARTICIPANTS

Alexander, B.	Boston, Massachusetts
Alkjaersig, N.	St. Louis, Missouri
Altschul, R.	Saskatoon, Canada
Baer, E.	Toronto, Canada
Ballerini, G.	Ferrara, Italy
Barkhan, P.	Philadelphia, Pennsylvania
Born, G. V. R.	Oxford, England
Boyle, A. J.	Detroit, Michigan
Brambel, C. E.	Notre Dame, Indiana
Braunsteiner, H.	Vienna, Austria
Brinkhous, K. M.	Chapel Hill, North Carolina
Conley, C. L.	Baltimore, Maryland
Cronkite, E. P.	Upton, L. I., New York
Cruz, W. O.	Rio de Janeiro, Brazil
Deutsch, E.	Vienna, Austria
Feissly, R.	Lausanne, Switzerland
Finch, C. A.	Seattle, Washington
Fulton, G. P.	Boston, Massachusetts
Gardner, F. H.	Boston, Massachusetts
Gross, R.	Marburg, Germany
Harrington, W. J.	St. Louis, Missouri
Hartmann, R. C.	Nashville, Tennessee
Hopkins, C.	Ann Arbor, Michigan
Horn, R. C., Jr.	Detroit, Michigan
Jackson, D. P.	Baltimore, Maryland
Jaques, L. B.	Saskatoon, Canada
Johnson, S. A.	Detroit, Michigan
Kinosita, R.	Duarte, California
Koppel, J. L.	Chicago, Illinois
Kramar, J.	Omaha, Nebraska
Krevans, J. R.	Baltimore, Maryland
Lüscher, E. F.	Bern, Switzerland
Miescher, P. A.	New York, New York
Monto, R. W.	Detroit, Michigan

LIST OF PARTICIPANTS — (Cont'd.)

Moore, C. V.	St. Louis, Missouri
Mustard, J. F.	Toronto, Canada
Odell, T. T., Jr.	Oak Ridge, Tennessee
Quick, A. J.	Milwaukee, Wisconsin
Rebuck, J. W.	Detroit, Michigan
Rosenthal, R. L.	New York, New York
Roskam, J.	Liége, Belgium
Seegers, W. H.	Detroit, Michigan
Sharp, A. A.	Oxford, England
Shinowara, G. Y.	New York, New York
Spaet, T. H.	New York, New York
Sprague, C. C.	New Orleans, Louisiana
Surgenor, D. M.	Boston, Massachusetts
Thomas, W. A.	Albany, New York
Tocantins, L. M.	Philadelphia, Pennsylvania
Troup, S. B.	Rochester, New York
Tullis, J. L.	Jamaica Plain, Massachusetts
Ulutin, O. N.	Istanbul, Turkey
Warren, H.	Philadelphia, Pennsylvania
Weissbach, H.	Washington, District of Columbia
Williams, W. J.	St. Louis, Missouri
Wintrobe, M. M.	Salt Lake City, Utah
Wissler, R. W.	Chicago, Illinois
Zucker, M. B.	New York, New York

ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL STAFF OF THE HENRY FORD HOSPITAL AND THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

RHEUMATOID LUNG DISEASE — REPORT OF A CASE WHICH DEVELOPED IN CHILDHOOD. G. L. BRINKMAN AND L. CHAIKOF. Am. Rev. Respiratory Dis. 80:732, 1959.

A case of rheumatoid lung disease is described in which roentgenographic abnormality was first noted at the age of six years, and death from pulmonary insufficiency resulted at the age of fifteen. At autopsy the lungs were markedly cystic but not emphysematous. There was extensive thickening of the alveolar septae. There were no distinct granulomas in any of the viscera. These changes are compared with the findings described in previously reported cases examined at autopsy.

THROMBOTIC THROMBOCYTOPENIC PURPURA OF LONG DURATION. S. F. CAHALANE AND R. C. HORN, JR. Am. J. Med. 27:333, 1959.

The clinical and autopsy findings in a case of thrombotic thrombocytopenic purpura of at least three and probably many more years' duration are reported. The presence of the characteristic vascular lesions material obtained three years before death and a positive serologic test for syphilis, later ascertained to represent a biologic false positive reaction, was discovered eighteen years before death. The literature on thrombotic thrombocytopenic purpura is reviewed and nineteen cases of prolonged survival are analyzed. It is pointed out that endocardial vegetations and occlusion of large blood vessels occur in cases of prolonged thrombotic thrombocytopenic purpura. The occurrence of disordered serum proteins is emphasized. It is suggested that platelet-derived fibrinogen may form the occlusive material in the arterioles and capillaries.

THE COMBINATION OF OXYGEN AND OPTIMUM FRACTIONATION IN RADIATION THERAPY OF ADENOCARCINOMA. L. A. DUSAULT, W. R. EYLER, AND G. D. DOBBEN. Am. J. Roentgenol. 82:688, 1959.

The results of irradiating spontaneous mammary adenocarcinoma in C_3H mice at the rate of 2,000 r per week to totals of 6,000 r or 8,000 r can be improved by allowing the mice to breathe 95 per cent oxygen and 5 per cent carbon dioxide during treatment; however, the advantage of breaking the total dose into fractions given three times a week as compared to daily treatment is still apparent. Although skin reactions appear to be the same, systemic effects, as measured by number of mice dying before completion of the treatment series, are increased by the gas mixture. These can be minimized by keeping the time of breathing it as short as possible.

DIVERTICULOSIS OF THE COLON. J. D. FOX AND J. L. PONKA. M. Arts & Sci. 13:16, 1959.

A brief resume of the problem of diverticulosis is presented. The various roentgen manifestations of this condition are illustrated and described. With an ever-increasing aging population in the United States, this condition is due to gain in frequency and prominence. About 3 per cent of the total population (5,000,000 persons) have diverticulosis, of whom 20 per cent (1,000,000) will have diverticulitis; 15 per cent

(150,000) of these will require operative treatment. The incidence of associated carcinoma in cases of diverticulosis and diverticulitis ranges from 6 to 10 per cent. Hence, the importance of carefully observing these patients since their symptoms may mask a silent carcinoma. Annual physical examination, including sigmoidoscopic and barium enema examinations, is advised for all persons having diverticulosis. Surgical treatment is advised only for the complications of diverticulitis and for all cases in which the roentgen diagnosis is equivocal with that of carcinoma.

IN VITRO STUDIES ON VITILIGO. F. Hu, R. P. Fosnaugh, and P. F. Lesney. J. Invest. Dermat. 33:267, 1959.

Data from cytological studies of 18 cases of vitiligo are presented. Included are 15 cases of typical vitiligo, 2 cases of congenital vitiligo and 1 case classified as pseudovitiligo. Our results appear to indicate that a favorable response to combined methoxsalen and ultra-violet light therapy depends on 3 basic mechanisms: 1) the presence of melanocytes which are capable of forming pigment (shown by dopa reaction), 2) the capacity for proliferation and/or migration of active melanocytes from adjacent pigmented areas to vitiliginous areas, and 3) the ability of epithelial cells to receive pigment from the active melanocytes (shown by silver technic). We would like to propose that a factor, equal in importance to the presence of melanocytes capable of forming melanin pigment, is operative in cases of vitiligo which fail to repigment following methoxsalen therapy. It is our opinion that this factor is closely related to the transfer of pigment from the melanocytes to the epithelial cells. We believe that the best method for demonstrating this ability of pigment transfer is by silver staining.

RELATIONSHIP OF THE ORAL ANTICOAGULANTS TO THE BLOOD COAGULATION MECHANISMS. S. A. Johnson, E. M. Priest, and M. J. Caldwell. Angiology 10:435, 1959.

The interrelations of prothrombin, Factor VII and PTC as affected by administration of the oral anticoagulants were investigated. When these three blood coagulation components were greatly reduced, fat soluble vitamin K_1 and water soluble vitamin K_1 were given to the patients. In spite of the very minimal differences in molecular configuration between these two compounds, different blood coagulation components appeared in the plasma and serum of the patient. Both vitamin K_1 compounds reduced the prothrombin time.

SUBCUTANEOUS FASCIOTOMY IN THE TREATMENT OF DUPUYTREN'S CONTRACTURE. A. P. Kelly, Jr. and R. H. Clifford. Plast. & Reconstruct. Surg. 24:505, 1959.

The subcutaneous fasciotomy is a procedure which offers negligible morbidity and worth-while correction of the deformity caused by Dupuytren's contracture. Its limitations are the frequency of recurrence of the deformity and the danger, inherent in a blind procedure, of injury to important structures. In young patients whose poor health precludes the more formidable fasciectomy, fasciotomy may bring appreciable relief and make one less aggravation in their lives. In instances of advanced contracture, it may release the fingers and allow mobilization and gradual stretching of the nerves, vessels and skin before a fasciectomy. For the patient with arthritic changes, the

atrophic, or the thick gross hand, fasciotomy should be considered. For middle-aged and elderly patients, it is a very satisfactory procedure which can be supplanted by a fasciectomy or repeated at the earliest evidence of recurrence. It is unquestionably the procedure of choice in older patients where the trouble is limited to a single bandlike deformity which shows no appreciable progression. The surgeon treating Dupuytren's contracture should carefully select his cases and use radical fasciectomy, partial fasciectomy, or fasciotomy, since each has a definite place in the proper treatment of this disease. It is only by a selective approach that we can give the patient the maximum benefit.

PRIMARY TENDON REPAIRS — A STUDY OF 789 CONSECUTIVE TENDON SEVERANCES. A. P. KELLY, JR. J. Bone & Joint Surg. 41-A:581, 1959.

Antibiotics, regional anaesthesia, and improved surgical facilities have made repair of tendon injuries easier and more attractive than previously. The results of this study indicate that crushing fractures and vascular insufficiency are still problems of great magnitude. It is incumbent upon the surgeon to recognize that the therapeutic advances in the past decade have not appreciably altered tissue reaction to trauma, and that the success of primary repair depends on proper evaluation of these factors as well as upon surgical skill. I have attempted to delineate the areas where primary repair is still a worth-while venture.

SIMPLIFIED TECHNIQUE FOR DIRECT VISION PULMONARY VALVOTOMY. C. R. LAM AND R. E. TABER. J. Thoracic & Cardiovas. Surg. 38:309, 1959.

It is proposed that open pulmonary valvotomy without hypothermia and without the pump-oxygenator is feasible and safe. Valvotomies have been carried out in 48 patients with a single mortality, which occurred in the youngest patient in the series. A second fatality occurred in a patient in whom pulmonic stenosis was absent, and in whom, at autopsy, two atrial septal defects were found. The operation is suitable only for pulmonic stenosis of the valvular type. Differentiation of this type from isolated infundibular stenosis and other congenital defects should be possible with the diagnostic techniques available to the cardiovascular team.

THE INCREASING INCIDENCE OF CARCINOMA OF THE THYROID IN A SURGICAL PRACTICE. J. M. MILLER, R. C. HORN, AND M. A. BLOCK. J.A.M.A. 171:1176, 1959.

A significant increase has been observed in the incidence of cancer in patients with thyroid malignancy. For the patients with a clinically undiagnosed thyroid cancer, qualitative and quantitative changes in patient population, greater number of operations for nontoxic nodular goiter, and improved clinical selection may account for most of the increase. The increased number of frank thyroid cancers is more difficult to explain on such grounds and suggests that the incidence of thyroid cancer in the general population is increasing. Physicians should regard the approach to preventive surgery of thyroid cancer as being in a dynamic state.

VENTILATORY RESPONSE TO INCREASING ARTERIAL CO_2 TENSION EMPLOYING A REBREATHING METHOD IN NORMAL INDIVIDUALS AND IN PATIENTS WITH CARDIAC DISEASE. H. G. PAULI, F. E. NOE, AND E. O. COATES, JR. J. Lab. & Clin. Med. 54:26, 1959.

Ventilatory response to an increasing arterial carbon dioxide tension was studied in 11 normal individuals and 20 cardiac patients, using a rebreathing method for gradual increase of CO_2 in the inspired air. A linear relationship between ventilation and arterial CO_2 tension was confirmed in both the normal individuals and cardiac patients. A diminution of the respiratory "threshold" for CO_2 with consequent increase in resting ventilation was found in all cardiac patients. Respiratory "responsiveness" to CO_2 was significantly diminished in the group of 7 patients with long-standing mitral stenosis. Studies after successful commissurotomy in two individuals suggested that these changes were reversible. Impaired mechanics of breathing may be responsible for the diminished responsiveness to CO_2 in individuals with long-standing mitral stenosis, as appears to be the case in some patients with primary hypoventilation states.

NEW CLINICAL CONCEPT OF SYSTEMIC LUPUS ERYTHEMATOSUS — ANALYSIS OF 100 CASES. C. E. Rupe and S. N. Nickel. J.A.M.A. 171:1055, 1959.

Increasing clinical awareness and study of the L. E. cell phenomenon have greatly expanded the understanding of the natural history of lupus erythematosus, revealing its prolonged, frequently benign course. The disease is much more common in men than has been previously reported. The natural history of lupus erythematosus as shown in this analysis suggests a classification of cases into chronic continuous, severe intermittent, and acute fulminating types. The prognosis in the individual case can be more accurately predicted if sex and type of organ involvement are taken into account. In this series a female patient with a butterfly rash, systemic involvement, and no arthritis had the worst prognosis and a male patient lacking evidences of renal involvement had the best. The frequent association of streptococcic infections preceding the onset of lupus erythematosus and repeated tissue response in the individual patient suggest a prophylactic possibility. Therapy with steroids may further change the prognosis in lupus erythematosus. At present the apparent increasing incidence of lupus erythematosus and the more frequent recognition of the earlier, milder case make a statistical appreciation of the effects of steroids and treatment difficult.

CLINICAL FEATURES OF CONTACT DERMATITIS DUE TO NEOMYCIN — REPORT OF CASES. H. Reynolds, J. F. Hildebrand, C. S. Livingood, and R. P. Fosnaugh. A.M.A. Arch. Dermat. 80:455, 1959.

Neomycin sensitivity is not common. However, the onset may be insidious and its recognition depends upon a heightened index of suspicion, rather than on the appearance of the classical signs of acute contact dermatitis. A total of 28 patients with neomycin sensitivity have been observed by us. Illustrative cases are described.

DIHYDROSTREPTOMYCIN DEAFNESS. G. E. Shambaugh, Jr., E. L. Derlacki, W. H. Harrison, H. House, W. House, V. Hildyard, H. Schuknecht, and J. J. Shea. J.A.M.A. 170:1657, 1959.

Cases of irreversible hearing loss attributable to dihydrostreptomycin are continuing to occur, usually without the knowledge of the prescribing physician, because of the latent period of from several weeks to as long as six months between administration of the drug and onset of the hearing loss. There is no known effective

treatment for this type of nerve deafness. In view of the ototoxicity of dihydrostreptomycin and, in particular, in view of the unique latent period, this antibiotic should be omitted from commercial combinations of antibiotics or, if it is included, its presence should be clearly indicated in the name.

PULMONARY ALVEOLAR PROTEINOSIS: REPORT OF THREE CASES. J. C. SIERACKI, R. C. HORN, JR., AND S. KAY. Ann. Int. Med. 51:728, 1959.

Three new cases of pulmonary alveolar proteinosis are reported. The histologic findings in biopsy material from the lungs are essentially identical and do not differ from those originally detailed by Rosen et al. The granular and crystalline inclusions in the proteinaceous intra-alveolar deposit that characterizes the disease are emphasized. Periodic-acid-Schiff-positive material, similar to the amorphous intrapulmonary deposit, was found in phagocytes in the supraclavicular lymph nodes of case 1. This study supports the concept that pulmonary alveolar proteinosis is a new disease, but a factor or factors common to all, or most, of the cases that might point to etiologic possibilities remain unidentified.

THE KIDNEYS IN SURGERY OF THE ABDOMINAL AORTA. D. E. SZILAGYI, R. F. SMITH, AND J. G. WHITCOMB. A.M.A. Arch. Surg. 79:252, 1959.

Certain problems related to renal physiology and anatomy that arise in surgery of the abdominal aorta are discussed. Some aspects of renal ischemia caused by cross clamping of the aorta and of the effects of angiographic contrast media on renal function are described. Instances of technical difficulties due to vascular anomalies (aberrant renal ateries and vein) are cited. Examples of the association of abdominal aortic aneurysm with horseshoe kidney and with ureteral obstruction are given. The following conclusions are offered: Although cross clamping of the aorta distal to the renal arteries has not been found responsible for any specific physiologic injury, its harmful potentialities must be kept in mind. The safe period of cross clamping of the aorta above the renal arteries has been found to be about 30 minutes for kidneys without manifest organic disease. In operative procedures requiring longer clamping, renal circulation must be maintained by some form of bypass. In aortography, the use of the least toxic contrast medium, in the smallest possible amount, with multiple exposure technique for the avoidance of repetition of injection is recommended. The usefulness of the pre- and postaortographic serum creatinine as functional index of renal function is stressed. In course of operative dissection of the abdominal aorta the possibility of the presence of accessory renal arteries must be remembered. Whenever the aortic dissection reaches above the level of the inferior mesenteric artery, the renal vein must be looked for, and if it is not found in its normal anatomical location must be sought in a retroaortic position. In dealing with horseshoe kidneys associated with an aneurysm of the abdominal aorta, bisection of the renal isthmus, suture of the cut ends, and resection of the separated kidneys allow the removal of the aneurysm in the routine manner. When an abdominal aortic aneurysm is complicated by ureteral obstruction, the resection of the aneurysm at the earliest safe moment is indicated.

OPERATION OF A BUBBLE TYPE PUMP-OXYGENATOR. R. E. TABER AND L. A. TOMATIS. S. Clin. North America 39:1539, 1959.

The assembly and operation of a bubble oxygenator of the DeWall-Lillehei type has been described, and some lessons learned in 300 perfusions have been reported. A compact stainless steel model of a bubble oxygenator is undergoing clinical trial. Some data about a practical disc type oxygenator are presented.

RELATIONSHIP OF ENAMEL HYPOPLASIA TO ABNORMAL EVENTS OF GESTATION AND BIRTH. W. F. VIA, JR. AND J. A. CHURCHILL. J. Am. Dent. A. 59:701, 1959.

Dental examinations and medical histories including prenatal, birth, and subsequent development information have been obtained for 312 children. A neurological examination revealed that 219 of these children had some form of cerebral disorder, whereas 93 had no evidence of cerebral disorder. Enamel hypoplasia was seen in 119 (54 per cent) of the children with cerebral disorders, whereas only 8 (9 per cent) of the normal children had enamel hypoplasia. The highest incidence of hypoplasia was seen in those children with choreoathetosis (100 per cent) and simple spastic diplegia (84 per cent). The children in the focal cerebral seizure and hemiplegia group had the least hypoplasia (38 percent). The abnormal factors of gestation and birth studied were: Rh incompatibility, maternal diabetes, toxemia of pregnancy, prematurity, breech presentation, twinning, cesarean section, labor in excess of 20 hours, intrapartum hemorrhage and placenta previa, and poor respiratory response at birth. Among the 312 children studied, enamel hypoplasia and one or more of the abnormal factors occurred in 93. A definite correlation was found between the time the factors occurred and the time the enamel hypoplasia was estimated to have occurred.